SCIENCE

JULY 28, 1950

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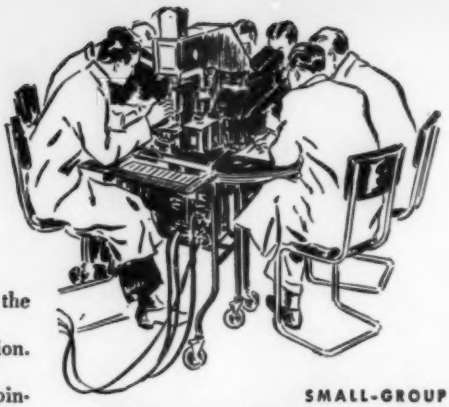

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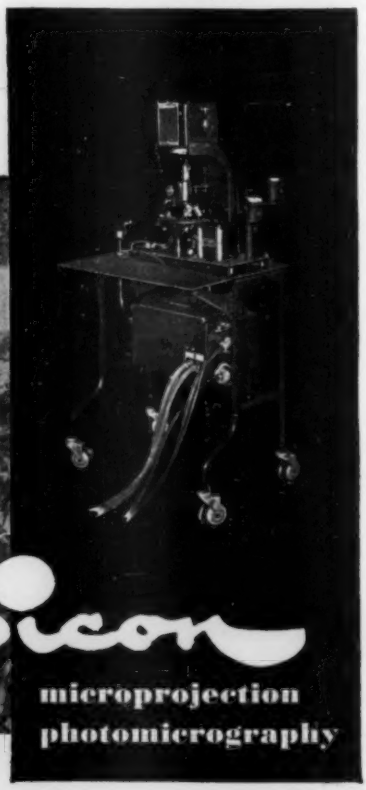

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What GENERAL ELECTRIC People Are Saying

H. N. HACKETT

Apparatus Department

MERCURY POWER CYCLE: [In a typical mercury-unit power plant] heat from the burning fuel is absorbed by liquid mercury within the tubes of the mercury boiler to form mercury vapor, which passes from the boiler to the mercury turbine, where it releases a portion of its energy to produce electric power. The vapor from the turbine is exhausted to the vacuum shell of the mercury condenser boiler. There it condenses and releases its heat of vaporization to water within the tubes. The liquid mercury is returned from the sump, or hot well, to the boiler by a mercury feed pump or by gravity, as the case may be.

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. . . The steam thus produced by the binary cycle is only slightly less in amount than it would be if the equivalent fuel were burned directly in a steam boiler. . . .

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Plant Engineers' Club,
Cambridge, Mass.,
April 20, 1950

★

A. W. HULL

Research Laboratory

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A new development of considerable promise is a rectifier or thyratron which substitutes caesium vapor for mercury as the conducting gas and simultaneously makes use of the condensed monatomic layer of caesium as cathode coating. . . . An interesting feature of this type of cathode is that its life is unlimited. Even more interesting is the voltage drop, which is the lowest known . . .

These tubes are only in the experimental stage.

A.I.E.E.
New York, N. Y.
February 1, 1950

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V. J. SCHAEFER

Research Laboratory

CLOUD SEEDING: Part of the benefit which may come from recent cloud-seeding experiments could be the *prevention* of rain or snow. Our investigations show that this could be brought about by intentional overseeding. By seeding supercooled or below-freezing clouds with excessive amounts of dry ice or silver iodide, we can bring about the formation of snow crystals which are unable to grow big enough to fall. By continuously seeding new supercooled regions as the cloud produces them, all—or a large part—of that cloud would either be dissipated or kept from growing into a thunderstorm.

There is even a possibility that, using the overseeding technique on huge areas of supercooled stratus clouds, we may produce snow crystals in such tremendous quantities that they could constitute holding reservoirs of snow crystals in the sky. These might later affect lower supercooled clouds, and in that way initiate widespread effects of a different pattern than would occur under natural conditions.

There is a distinct possibility that dangerous aircraft-icing conditions caused by supercooled clouds might be eliminated or reduced along major airline routes or in the vicinity of airports by converting the clouds to snow crystals. In a similar manner, ground fog, if it is sufficiently supercooled, might be cleared by seeding.

WGY Science Forum,
March 8, 1950.

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Astrochemical Problems in the Formation of the Earth

Wendell M. Latimer

Department of Chemistry, University of California, Berkeley

THE ASSUMPTION that the earth was formed by condensation of a cold cosmic cloud appears to offer a more satisfactory explanation of its composition than does the assumption that the earth was condensed directly from a mass of hot gas.[1] On the latter assumption, it is difficult to account for such facts as the presence of water but the absence of quantities of the noble gases, the constant isotopic composition of matter in the earth and meteorites, and the presence of both metallic and oxidized iron.

The composition of the earth is essentially that of the solid particles which may be presumed to be present in a cold cosmic cloud. The process of condensation then must separate these particles from the large excess of gaseous material and bring them together to form a mass with the characteristics which the geologists ascribe to the earth in its initial state. The essential features of this geological picture[2] are a central core of iron (with about 8 percent nickel), surrounded by a mantle of magnesium and iron meta- and orthosilicates and an outer layer of basalt. In the initial stages there was no surface water, no atmosphere, and no granite masses on the surface—that is, no continents.

Initial Composition of the Cosmic Cloud

Brown (*3*) has summarized the data on the relative abundances of the elements in the sun and stars. His values are given in Table 1. At a temperature of not more than a few hundred degrees Kelvin, a cosmic cloud formed from the elements with these abundances would consist of solid particles and gases. The weight of the gaseous materials was several hundred times that of the solids. Chemical thermodynamics permits definite conclusion as to the compounds present in both the gas and the solid particles. The more important materials are summarized in Table 2, and the thermodynamic data relating to their stabilities at 298° K are given in Table 3. It may be presumed that the average composition of the solid particles was constant throughout the cloud. Because of the large excess of hydrogen and water, stabilities are determined by oxidation-reduction potentials relative to these substances. Oxygen will be present either in very stable solid oxides or as water; the nitrogen as nitrides or ammonia; and the carbon as carbides or methane. A very important problem is that of iron and ferrous oxide.

$$FeO + H_2 = Fe + H_2O, \quad \Delta F_{298} = 3.8 \text{ kcal};$$

$$k = \frac{P(H_2O)}{P(H_2)} = 1.6 \times 10^{-3}.$$

From the relative abundances of H and O (Table 1) the ratio $\frac{P(H_2O)}{P(H_2)}$ in the gas phase is 5×10^{-4}. Hence, at 298° K the equilibrium favors the reduction and FeO is unstable. However, if the FeO is combined with SiO_2 to form $FeSiO_3$, the equation becomes

$$FeSiO_3 + H_2 = Fe + SiO_2 + H_2O, \quad \Delta F_{298} = 12 \text{ kcal};$$

$$k = \frac{P(H_2O)}{P(H_2)} = 10^{-7}.$$

Hence, $FeSiO_3$ (also Fe_2SiO_4) is stable at 298° K and would remain so below 600° K. This becomes an extremely significant fact, since the amount of oxidized iron will depend upon the SiO_2 available for the formation of the iron silicates. $MgSiO_3$ and Mg_2SiO_4 appear to have equal or greater stability than $FeSiO_3$ and Fe_2SiO_4, and the amount of oxidized iron should therefore depend upon the relative abundance of Mg and Si. From Table 1 the relative abundances are found to be 8870 and 10,000, respectively. The meteoric silicates are approximately 45 percent *ortho*. Hence 8870 Mg would tie up only 6865 SiO_2 and leave 3135 SiO_2 for the formation of iron silicates. Assuming the percentage of the ortho and meta silicates is the same for both Mg and Fe, the ratio of magnesium to oxidized iron is then $\frac{6865}{3135}$, or 2.2. Brown (*2*) has given the weight percentages of magnesium and oxidized iron in the silicate phase of meteorites as 16.62 and 13.23, respectively. The corresponding atomic ratio is 2.9, which is in approximate agreement with the value calculated above, and if a correction is made for the SiO_2 combined with Ca, Na, and K, the agreement would be even better.

[1] This point of view was expressed by Harold Urey, Harrison Brown, and the author at the Rancho Santa Fe Conference on the Formation of the Earth, held under the sponsorship of the National Academy of Sciences, January 23–25, 1950.

[2] The geologists present at the Rancho Santa Fe Conference were in general agreement on the broad aspects of this picture.

TABLE 1

COSMIC RELATIVE ABUNDANCES

Element	Abundance*	Element	Abundance
H	3.5×10^9	A	130–2,000
He	3.5×10^7	K	69
C	80,000	Ca	670
N	160,000	Sc	0.18
O	210,000	Ti	26
F	90	V	2.5
Ne	$10^4 - 10^5$	Cr	95
Na	462	Mn	77
Mg	8,870	Fe	18,300
Al	882	Co	99
Si	10,000	Ni	1,340
P	130	Cu	4.6
S	3,500	Zn	1.6
Cl	190	Ga	0.65

* Atoms per 10,000 atoms of Si.

Condensation of the Earth Cloud

It may be assumed that the initial diameter of the earth cloud was less than half the distance between the orbits of Mars and Venus, or 5×10^{12} centimeters. During the condensation of the cloud under gravitational forces to form the earth, two important processes occurred: one, the loss of the gaseous material and, two, a concentration of the iron particles toward the center of the mass.

The gaseous material escaped even if the total mass (gas plus solids) was several hundred times the present mass, since the gravitational force at the surface of the mass was insufficient to hold the gases until the diameter was greatly reduced. The problem cannot be stated exactly without a knowledge of the surface temperature. The surface toward the sun would be heated, and the gravitational heating would be considerable, as will be discussed later. A temperature of not less than 400° K (*1*) will be taken as a rough estimate (*5*). At that temperature a gas with a molecular weight of 40 would escape until the radius was reduced at least 100 times. It seems probable, then, that in the condensation from a radius of about 2.5×10^{12} cm to 6.37×10^8 cm, even the heavier gases were lost and the earth as originally formed was without an appreciable atmosphere. This is in agreement with the conclusions of Brown (*1*).

During the major portion of the condensation process the particles are "falling" through an appreciable concentration of gases. By Stokes' law the velocity of fall is given by the expression

$$V = \frac{2a^2(d - dm)G}{9\zeta}$$

where a is the radius of the particle, d is density, dm the density of the medium, G the acceleration of gravity, and ζ the viscosity coefficient. The density of the particles varies from about 8 for the iron-nickel to 3.5 for olivine and 2.9 for the basalt. Thus from the density difference, the iron-nickel particles would fall with a velocity two to three times that of the silicate mineral. However, it is likely that the iron particles with a simple crystal lattice grew to much greater size than the more complicated silicates, and, since the velocity is proportional to the square of the radius, this factor could have produced large differences in velocities. An iron particle with $a = 10^{-2}$ cm would fall three million times faster than a silicate particle with $a = 10^{-5}$ cm. It seems likely, then, that the process of condensation concentrated the metals toward the center of the earth and also concentrated the heavier orthosilicates around the metal core with the lighter basalt nearer the surface.

TABLE 2

PRINCIPAL SUBSTANCES PRESENT IN INITIAL CLOUD

Gases	*Solids (continued)*
H_2	Oxides or their compounds of Fe, and all elements more electropositive than Fe, e.g., Fe_2SiO_4, $MgSiO_3$, $Ca_3(AlO_3)_2 \cdot nH_2O$, $Al(OH)_3$
H_2O	
CH_4	
NH_3	
He, Ne, etc.	Nitrides, Fe_3N, etc.
Solids	Carbides, Fe_3C, etc.
Metals (Fe and all less electropositive elements)	Halides, CaF_2, NaCl, NH_4Cl, etc.
	Sulfides, FeS, PbS, etc.

TABLE 3

ΔH, ΔF, AND S AT 298° K

Compound	ΔH (kcal)	ΔF (kcal)	S (cal/deg)
H_2	0	0	31.21
NH_3	−11.04	−3.976	46.11
CO	−26.41	−32.81	47.3
H_2O (gas)	−57.80	−54.63	45.10
H_2O (liquid)	−68.31	−56.69	16.71
SiO_2	−205.4	−192.4	10.0
FeO	−63.7	−58.4	12.9
CO_2	−94.45	−94.26	51.06
CH_4	−17.89	−12.14	44.50
$FeSiO_3$	−276	−258.8	(22)
Fe_2SiO_4	−343.7	−319.8	35.4
Fe_3C	5.0	3.5	25.7
Fe_3N	−0.9	2.6	24.2
TiN	−73.0	−66.1	7.20
TiC	−54	−53	5.8

It is possible that the moon was formed in the later stages of condensation. When the diameter was several times that of the final value, tidal waves in the "loose material" may have been sufficient to cause a rupture. Since the heavier iron and mineral particles had already been concentrated toward the center of the earth, the composition and density of the moon formed at that stage would correspond to that of the outer layers of the earth.

The most difficult point to understand in the condensation process is how the enormous gravitational energy could have been dissipated to such an extent that the earth condensed comparatively cold. The total gravitation energy of condensing the solid particles is 2.6×10^{39} ergs, or 10^4 calories per gram. In

the early stages of the condensation the escaping gases certainly carried away large amounts of energy, but in the final stages the particles are presumably falling in a near vacuum, and a body falling from only a few miles out from the earth's surface would acquire a kinetic energy sufficient to heat it to several thousand degrees.

The answer lies in the fairly high viscosity coefficient of a cloud of small dust particles even with no gas present and the low terminal velocity which the particles of the cloud would therefore possess. From kinetic theory the viscosity coefficient of a system of particles with a thermal velocity V_k, mass m, and radius a, is:

$$\zeta = \frac{mV_k}{16\pi a^2}$$

Assuming the thermal velocity for a temperature inside the cloud of 600° K, which is below the temperature at which the hydrated silicates would lose water, the value of the viscosity coefficient for particles of $a = 10^{-5}$ cm is about 10^{-4}. Using this value to calculate the terminal velocity of particles of this size and density $\rho = 3$, one finds

$$V_t = \frac{2a^2\rho g}{9\zeta} = 6.5 \times 10^{-4} \text{ cm/sec.}$$

In view of this low velocity of fall of the cloud, one feels justified in equating the rate of decrease of gravitational energy to the decrease in energy by radiation:

$$\frac{Gm_0m_1dR}{{R_0}^2} = 4\pi r^2 cT^4 dt,$$

where G is the gravitational constant, m_0 the mass of the earth, m_1 the mass of the particle, R_0 the radius of the earth, c the radiation constant, and t is time. Substituting $V_t dt$ for dR, and simplifying:

$$\frac{T^4\zeta}{a^2\rho^2} = \frac{2}{27}\,\frac{G^2{m_0}^2}{c{R_0}^4} = 1.2 \times 10^{10}.$$

This equation assumes that each particle is radiating into space—an assumption that is, of course, not true. And a knowledge of the coefficient of opacity is required for an exact solution (*4*). However, a cloud of particles with radii less than 10^{-3} cm would apparently not acquire a high temperature in slowly falling to the earth's surface.

Heating of the Earth

If the earth was formed as a comparatively cold mass, we must next consider the problem of how its present internal temperature was attained. A calculation of the energy produced by the radioactivity of K^{40} shows that this source of energy is very appreciable. The calculation, using Brown's (*3*) values for the abundance of potassium, follows:

Abundance of K, 0.12 percent.

Total K = $0.0012 \times 6.1 \times 10^{27} = 0.73 \times 10^{25}$ grams.

K^{40} at present, 0.012 percent of total K = 0.88×10^{21} grams.

Half-life of K^{40}, 1.5×10^9 years, uncorrected for K-capture.

Assumed age of earth, 3×10^9 years.

Amount of K^{40} at time of earth's formation, $4 \times 0.88 \times 10^{21} = 3.52 \times 10^{21}$ grams.

Maximum energy from K^{40} β^-, 1.3 Mev. Mean energy from K^{40} β^-, 0.5 Mev. Energy corrected for K-capture, 0.6 Mev.

Heat produced by reduction of K^{40} from 3.52×10^{21} grams to 1.76×10^{21} grams $= \frac{1}{40} \times 1.76 \times 10^{21} \times 0.6 \times 10^6 \times 2.3 \times 10^4 = 6.1 \times 10^{29}$ calories.

Assuming all K is in the mantle, and weight of mantle is 50 percent of weight of earth:

$$\text{Heat per gram of mantle} = \frac{6.1 \times 10^{29}}{3.05 \times 10^{27}} = 200 \text{ cal/g.}$$

Specific heat of mantle material 0.2 cal/g.

$$\text{Rise of temperature } \frac{200}{0.2} = 1000° \text{ C.}$$

Thus in the first 1.5×10^9 years the K^{40} alone would have increased the temperature of the mantle to about 1300° C. If most of the potassium were concentrated in the outer basalt layer, this region could easily attain temperatures above 2000° C. In addition to the K^{40} effect, the heat liberated by the uranium radioactive series must be considered. A calculation for the decomposition of U^{235} to lead over the period 2.8×10^9 to 9×10^8 years ago gives as the heat produced 3×10^{29} calories, or about 50 percent of that liberated by K^{40}, and a summation of the heat from all the radioactive series would probably add another 25 percent.

Formation of the Atmosphere

As the temperature of the interior of the earth increased, various chemical reactions occurred, a number of which liberated the materials now forming our atmosphere.

1. A portion of the basalt decomposed to give the granite, which rose to form the continents, and dunite, which tended to sink to lower levels.

2. The hydrated silicates and aluminates were broken down with the liberation of steam, which rose to the surface to form the present water.

3. Ferrous oxide oxidized many of the carbides to form CO_2, e.g.,

$Fe_3C + 2FeO = 5Fe + CO_2$; $\Delta F_{298} = 19{,}000$ calories.

The free energy of the reaction is positive at 298° K, but the entropy of the reaction is about 50 cal/deg; hence, the reaction will go above 500° K.

4. Since the excess of hydrogen has been lost, the newly formed steam will oxidize more Fe:

$Fe + H_2O = FeO + H_2$; $\Delta F_{298} = -3800$ calories.

Most of the free iron which remained in the outer layers was thus oxidized.

5. Nitrides were hydrolyzed by steam to ammonia:

$2Fe_2N + 3H_2O = 3FeO + Fe + 2NH_3$; $\Delta F_{298} = -21{,}900$ calories.

Free Fe_2N would have been unstable in the original cloud, but considerable quantities doubtless were present as a dilute solution in Fe. The same is true of the Fe_3C. The ammonia would decompose in the hot regions into N_2 and H_2. Urey has suggested that ammonia was also liberated from NH_4Cl by reaction with basic oxides.

Later Changes in the Atmosphere

At this stage the atmosphere consisted of water vapor, carbon dioxide, nitrogen, hydrogen (which was slowly lost from the gravitational field), and possibly some ammonia. Photochemical changes have added our present supply of oxygen. The hydrolysis of the complex silicates freed basic oxides such as CaO and MgO, which absorbed large amounts of the CO_2. Any ammonia has been oxidized to nitrogen by the oxygen. Argon has been added by the β-decomposition of K^{40}.

The Origin of the Earth Cloud and the Other Planets

Since the earth cloud of gas and dust particles was unstable with respect to its ability to retain the gas, it must have been formed by the breakup of a larger cloud, and the other planets were doubtless formed in the same manner from the same cloud. The characters and origin of the larger cloud are open to considerable conjecture. Von Weizsäcker (*6*) has discussed the mechanics of the condensation of a cosmic cloud to form a sun and planets. The problem has also been treated by Whipple (*7*), and the following postulate is somewhat along the lines suggested.

In the process of condensation of the cosmic cloud (*A* in Fig. 1), it may be assumed that the sun acquired a small diffuse companion (*B*, Fig. 1), either in the original condensation process or from one or two smaller neighboring clouds. This diffuse companion would have condensed to a small companion star, but its rotational and orbital velocities were so large that it was distorted (*C*, Fig. 1), and broke up into smaller clouds (*D*, Fig. 1). The minor planets were formed from the tail or winglike portion of the

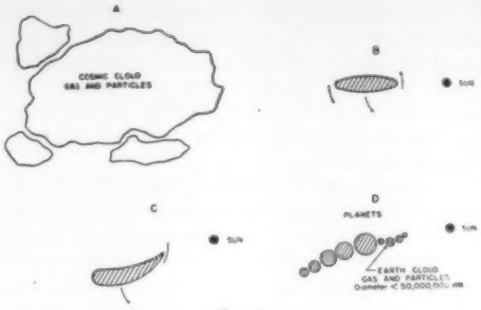

FIG. 1.

cloud, and the major planets from the more massive portion. All the minor planets should have condensed to bodies without an atmosphere. The major planetary clouds were all of sufficient mass to hold the lighter gases, and their composition should approximate the total composition of the initial cloud, i.e., the sum of the gases plus the solid particles. Pluto, on the other wing, is presumably more like the earth, and any more distant planets would be expected to be smaller.

The suggested formation of the earth cloud from a diffuse companion of the sun is not essential to the arguments with regard to the development of the earth in its present state; however, the hypothesis does give a reasonable picture of the distribution of mass among the various planets, and some picture is required that gives a common origin of all the solid material of the earth and meteorites to account for their constant isotopic composition.

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August Krogh: 1874–1949

Cecil K. Drinker
Falmouth, Massachusetts

AUGUST KROGH was born in Grena, a small town in Jutland, where his father owned a brewery. His mother took care of his early education, and he went to school rather late. He became a Master of Science in zoology in 1899 and received his Ph.D. in zoology from the University of Copenhagen in 1903.

After 1899 he worked as assistant at the Physiological Institute under Christian Bohr, still known to us in physiology for his fundamental discovery in respiration, the "Bohr Effect," and in recent years known to us more generally through his distinguished son, Niels Bohr. It was natural that Krogh's thesis dealt with a respiratory problem, "On the Skin and Pulmonary Respiration of the Frog." It was published in the *Skandinavisches Archiv f. Physiologie*, and has proved a permanent addition to our knowledge.

At that time Christian Bohr was the chief champion of the theory that gases, especially oxygen, were secreted by the lung epithelium into the blood. Krogh accepted this theory, but he and Bohr agreed it was based on inconclusive evidence. In 1905, using methods of analysis developed mainly for work in insect physiology, he determined to search for more decisive evidence through studies of the tension of O_2 and CO_2 in the blood and in the alveolar air. This work was continued until 1910, much of it in collaboration with his wife, Marie Krogh, whom he married in 1905 while she was a medical student. The results led steadily away from the secretory theory and to convincing evidence that gas transport depended upon simple diffusion.

In 1908 he became lecturer in physiology at the University of Copenhagen and in 1910 was given a small laboratory of his own. This he named The Laboratory of Zoophysiology, changing from the original designation, Animal Physiology, in order to reduce attention from the antivivisectionists. The laboratory received in 1910 was occupied by Krogh and his pupils until 1928, when he moved into the beautiful new building which was given to the University of Copenhagen by the Rockefeller Foundation principally because of his accomplishments in physiology and in the fundamental understanding of medical problems.

Krogh's finest work was done in the original laboratory, although until his death he was steadily active with his own hands and at any time might have again been found in the midst of epoch-making discoveries. He had an abiding love for experimental work, which asserted itself wherever he was.

The first laboratory was in part of a solid block of Danish houses at Ny Vestergade 11. The quarters had been the university laboratory of bacteriology. Structurally, they offered exactly the same possibilities for efficiency and comfort as would be supplied by a large American house in the center of a heavily populated block in any of our large cities.

Krogh and his family lived on the two upper floors, thus having a proximity to the laboratory he felt most important. Indeed, I remember he told me in 1926 he would not move into the new quarters which were being built unless they included a place to live. This feeling did not arise from motives of comfort or economy. It was the expression of his total immersion in the affairs of the laboratory and his unwillingness not to be able to go and come at any time.

The laboratory was very small. No one, the Professor included, had a room to himself. Paul B. Rehberg, Krogh's assistant during my year in Copenhagen, did that winter occupy a small room alone, but the door was never shut, and at any time it was clear someone might go in with him.

In 1926–1927 A. N. Richards, Edward D. Churchill, and I occupied a room together. There was a large table in the center and old-style laboratory desks were on one side, together with a sink supplying cold water. Hot water was heated over an old coal stove in one corner, where a kettle was always in place. If we wanted more hot water or wanted it in a hurry a gas ring was available. We washed our own glassware and at one time I had a lot of it. I cannot remember we suffered from this!

In this room and two other small ones adjoining it, Krogh initiated, and to a large degree carried out, his observations upon the capillaries, for which he received the Nobel Prize in 1920. This work had somewhat the same sort of impetus as his first researches on gas transport. He was examining the diffusion of oxygen out of blood and into muscle. Gradually he became aware that, given the capillary area apparently available at rest, it was impossible to obtain the amounts of oxygen used by the muscle at work. In

explanation he thought that capillaries in working muscle not only dilated but that vessels, closed during rest, became patent, so that the capillary surface for gas exchange was enormously increased and the muscle was enabled to work in an environment wholly suited to activity. The many investigations through which this first demonstration led Krogh and his pupils do not require comment. In the main, they were summarized in his Silliman Lectures at Yale in 1922 and published in his monograph, "The Anatomy and Physiology of the Capillaries."

The man himself was modest and informal. He possessed the simple directness of thought and approach that accompany absolute honesty. I remember how each morning about ten he drifted noiselessly downstairs in his old carpet slippers and entered the laboratory. He came to each of us in turn, always shook hands, and then at once began to talk of our work, which was never out of his mind. He had questions for us and suggestions as to progress and he was delighted if we came forward with posers for him. The experiments in progress were more or less integrated, but each worker owned his problem, and, although Krogh usually suggested the research, he never allowed his name to appear on the paper unless he had actually taken part in the work. The list of his publications is formidable, extending through 48 years of almost unbroken activity. He abhorred "directors" of laboratories, believing that a man responsible for a laboratory had the privilege of acting as a sort of enzyme to help produce results but had no place as the principal figure in the task.

After 1910, when Krogh became independent in the Ny Vestergade laboratory, I have been able to count over twenty Americans who worked in the new laboratory, and of this number all except two became professors or heads of departments. With the possible exceptions of Sir Joseph Barcroft and Sir Thomas Lewis, I believe August Krogh dealt with more American students than any of his contemporaries. His personality and his influence were never lost to us, nor did he ever cease to be interested in what each of us was doing.

He spoke and wrote English almost perfectly. On his visits to my house I remember how assiduously he went through *The New Yorker,* never permitting a phrase to escape him. His sense of humor was keen, and he appreciated the American idiom. He read many American books and was devoted to Mark Twain, William Prescott, and Francis Parkman—the last two fed his interest in archaeology. During the last twenty years of his life Krogh came frequently to this country. As he entered our laboratories, all were conscious of his quiet friendliness, and of the readiness with which he listened to and examined the work being done. He had great sagacity and directness in planning experiments. He was an excellent technician and deviser of apparatus, but he was completely ruthless in making it as simple as possible. The beauty of mechanical things attracted him, but he considered refinements in apparatus unessential to the problem at hand to be expressions of weak intellect, and they never interested him.

From 1901 until about 1917, Krogh's investigations were largely in the field of respiration and gaseous metabolism, and students who went to him represented this aspect of physiology. His interest, however, was inclusive, and he never forgot the breathing and energy metabolism of insects, which had intrigued him at the beginning of his career and which, after he retired from his professorship in 1944, became his entire occupation in the small private laboratory he had constructed.

Krogh's absorption in physiology did not preclude other activities. He was a faithful and influential member of the university faculty and of the Danish Royal Society. In March, 1949, a bit before his final illness set in, he wrote me describing the sort of action which was characteristic of him, though unexpected in so quiet and contained a man.

> I caused a great stir in Denmark by resigning on Jan. 14th from our Roy. Soc. because I could not persuade them to go in actively to improve conditions of scientific work in this country. The whole of the Danish press commented favorably on this step and I feel sure that in the long run it will prove useful. At least it has shown the authorities that if they want to do something positive public opinion will be behind them.

In 1922, when in this country, he learned all he could about the production of insulin and on returning to Copenhagen he joined Hagedorn in the founding and construction of the Insulin Institute, which supplied and continues to supply the drug to Scandinavia and much of Europe, besides notably advancing research on diabetes.

The second world war was an incredible experience to him. He was a warm admirer of German science and had many German students. The intellectual descent of Germany into Nazism was to him impossible. When the Nazis took Denmark and Norway and attempted to impose an ignorant dictatorship upon these highly cultivated and free-thinking people, he joined his friends in constant, silent revolt. Had he been younger his participation would certainly have been active, and he would have suffered physically as did his associate Rehberg. In October, 1944, Krogh wrote:

> I don't think I have had a word from you after the terrible April 1940, but I have taken the opportunity to

write you at least once during a visit to Sweden. Now again I am here, and this time I shall have to stay until Denmark is liberated. About the middle of June I had an intimation of imminent danger and had to go "underground," which in my case meant going about in North Zealand on my bicycle and staying for periods not too long with various friends and relatives. I am sorry to say that I had not really deserved this honour, having done very little towards the cause, and I fear it was only because I was considered "prominent" that I might be worth "liquidating." I had arranged to spend the summer at a small and secluded limnological laboratory, when I received word from Stockholm that my son-in-law, Christer Wernstadt, was very seriously ill, and could I come. After deliberation with well-informed persons it was decided that I should apply for regular visas and try to come "legally" to Sweden, and this succeeded in such an incredibly short time that there is a strong suspicion that the German official in question knew of the danger and wished to be helpful. Anyway I arrived safely on July 9th.

In addition to the Nobel Prize, August Krogh received honorary degrees and memberships in scientific societies from many parts of the world. It is our pleasure that he was one of those given the doctorate at the Harvard Tercentenary in 1936, and thus, in addition to having had a hand in the training of eight full professors at Harvard, he appears upon the rolls of the university.

Those who knew him will never forget this man and those not so fortunate will ever gain inspiration from his clear-sighted and classically simple observations.

Technical Papers

A Limitation on the Ultracentrifuge Separation-Cell Technique[1]

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The ultracentrifuge separation cell has been increasingly utilized in recent years in investigations of the biological activity associated with certain high molecular weight solutes (*2*, *3*, *8*). In this analytical cell a porous plate overlaid with filter paper divides the cell into two compartments. This permits occurrence of analytical sedimentation, observed by the usual optical methods, but prevents remixing of the contents of the two chambers at the end of an experiment. The sedimenting boundary of the characteristic substance suspected of being biologically active may then be brought to various known positions in the cell in different experiments, the rotor rapidly decelerated, and the biological activity remaining in the top compartment of the cell determined. If one finds that the residual activity is exactly proportional to the amount of the characteristic substance remaining in the top chamber, one has apparently eliminated the possibility that impurities of either significantly smaller or significantly larger sedimentation rates than that of the characteristic substance are the active principles.

It is not clear, however, that this conclusion is justifiable under the following set of circumstances. Let us suppose that we have a solution containing a characteristic substance (hereinafter referred to as CS) in relatively high concentration (1–10 mg/ml), together with a very small amount of an active principle (AP), which is responsible for all of the particular biological activity in this mixture. Let us further assume that the sedimentation constant of the AP is larger than that of the CS. Upon performing a separation-cell experiment with this mixture, might not the small thermal gradients occurring during sedimentation, or the vibration of the rotor, be sufficient to disrupt the AP sedimenting boundary, since the density gradient across this boundary would be very small? Furthermore, might not the AP then be distributed relatively uniformly ahead of the stable CS boundary, but not be convectively transported across it? Under these circumstances, the gradient of activity through the cell might very nearly coincide with the gradient of concentration of the CS, creating the illusion that the CS is the active principle.

In order to examine this problem, which is of considerable interest, for example, in the study of plant viruses (*2*), we must know more about the behavior of very dilute solutions in the ultracentrifuge. The usual optical methods for determining sedimentation rates are not sensitive enough to permit the study of solutions of concentration lower than about 0.1 mg/ml. Bacteriophage particles, however, offer the possibility of studying this problem. Their concentrations can be followed accurately by infectivity measurements in extremely dilute solutions. Despite the fact that some of these bacteriophages have a morphology which makes the term "molecules" inapplicable to them, they behave like protein molecules in the usual sedimentation and diffusion

[1] Presented before the American Chemical Society at the meeting in Philadelphia, April 9–13, 1950.

[2] Postdoctoral research fellow, National Institutes of Health, United States Public Health Service.

[3] Contribution No. 1427.

experiments. Sharp *et al.* (7) investigated relatively concentrated T2 *Escherichia coli* bacteriophage solutions in the ultracentrifuge by the ultraviolet absorption optical method. Polson (4) studied the diffusion behavior of T4 and T3 bacteriophages, and showed that the tail of the former particle does not significantly affect its diffusion properties. Both particles obey the Einstein equation for diffusion reasonably well in relatively concentrated solutions. A review of the molecular kinetic properties of bacteriophages was recently published in this journal (6).

Our experiments were performed with T2 bacteriophage. The raw bacterial lysates, which were prepared in M-9 synthetic medium, were concentrated and purified by differential centrifugation. The infectivity of the resulting preparation was of the order of $10^{-15.0}$ g N/infective particle.

The ultracentrifuge used in these studies was designed and built in the Chemistry Department of this institute. A detailed description of it will appear elsewhere.

Various concentrations of bacteriophage in cacodylate buffer, pH = 6.9, $\mu = 0.1$, were examined by the separation-cell technique. In experiment U-46, with a solution containing 0.5 mg/ml, the sedimentation was followed by the usual Philpot Schlieren optical method. Two peaks were recorded with sedimentation constants of about 1,000 and 700 *S*. This two-peak phenomenon was observed by Sharp *et al.* (7). After 2,300 sec of sedimentation at 149.3 rps the slower peak was well past the porous plate partition. All the experiments, the results of which are given in Table 1, were run for the same time at the same speed.

TABLE 1

Exp. No.	Original solution		Solution removed from top chamber	Residual activity %
	Titer T2/ml	Conc. mg/ml	Titer T2/ml	
U-46	6.0×10^{11}	5.0×10^{-1}	4.4×10^{9}	0.7
U-45d	1.1×10^{10}	9.1×10^{-3}	1.8×10^{8}	1.6
U-45c	8.0×10^{8}	6.6×10^{-4}	5.1×10^{7}	6.4
U-45b	6.1×10^{7}	5.1×10^{-5}	2.2×10^{6}	3.6
U-45a	6.5×10^{5}	5.4×10^{-7}	2.8×10^{4}	4.3

A calibrated thermocouple with one junction situated in the slip stream of the rotor indicated that the temperature of the rotor changed by no more than 0.2° during the runs. An air pressure of 5×10^{-2} mm Hg was maintained in the centrifuge chamber and no cooling of the chamber was necessary at this low speed. Control experiments indicated that the bacteriophage suffered no change in infectivity upon standing at room temperature in the separation cell for comparable times.

These experiments indicate that an increasing proportion of the bacteriophage particles is left in the top compartment upon sedimenting more dilute solutions under identical conditions. This could not be due to a displacement of the mean ordinate of the sedimenting boundary since, in the absence of disturbing influences, the sedimentation constant should increase with dilution (1). The results suggest rather that convection currents increasingly perturb the concentration distribution about the mean ordinate of the boundary with increasing dilution.[4] There does not appear, however, to be a concentration at which an abrupt change takes place in the stability of the boundary.

TABLE 2

Exp. No.	TMVP Boundary position (see text)	Bacteriophage titers $\times 10^{-6}$		Residual activity %
		original solution	residual solution	
U-5a	0.50	3.8	0.15	4.0
U-7	0.88	2.1	0.033	1.6
U-6	1.25	2.1	0.024	1.1

A second set of experiments was performed with mixtures of 5 mg/ml of tobacco mosaic virus protein (TMVP) and about 10^8 T2 bacteriophage/ml in the cacodylate buffer used previously. The TMVP boundary, moving at a rate equivalent to about 200 *S*, was sedimented to various positions in the cell. In experiment U-5a, the boundary was taken 0.50 of the distance from the top of the cell to the porous plate partition; in experiment U-7, 0.88 of this distance; and in experiment U-6, about 0.25 of this distance *past* the partition. The contents of the top compartment were then analyzed for bacteriophage activity. Control experiments indicated that TMVP has no effect on bacteriophage infectivity.

The results of these experiments are listed in Table 2. The fact that the titers in experiments U-7 and U-6 of the solutions removed from the top compartment were only slightly different demonstrates that no sharp change in bacteriophage concentration occurs across the slower moving TMVP boundary. In other words, the convective transport of the bacteriophage particles is not seriously affected by the presence of the stable, slower moving boundary.

If the results obtained in these experiments are of general validity, we may summarize them as follows:

a) Thermal gradients and other perturbing influences such as vibration occurring during analytical ultracentrifugation are apparently capable of disturbing but not completely disrupting the sedimenting boundaries of very dilute solutions.

b) The convective transport of the dilute solute is not significantly affected by the presence of a stable, slower moving sedimenting boundary.

c) It should therefore be feasible to eliminate in the following manner the possibility that a certain biological activity is carried by an impurity with a higher sedimentation constant than that of the CS. The CS boundary may be sedimented about 0.5 of the distance to

[4] The large increase in the apparent diffusion rates of bacteriophage particles in the concentration range from 10^9 to 10^{13} particles/ml, observed by Polson and Sheppard (5), may also be interpreted as due to an increased convective transport of the solute in more dilute solutions.

the partition in the separation cell, and the contents of the top compartment analyzed for activity. These contents may then be diluted to a volume sufficient to refill the cell and the above experiment repeated. After several such cycles, if the ratio of activity to the amount of the CS left in the top compartment remains constant, then it may be concluded that no substance, whatever its concentration, sedimenting significantly faster than the CS, is active.

Similar considerations should apply in electrophoresis separation-cell experiments.

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Germaniferous Lignite from the District of Columbia and Vicinity[1]

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A unique accumulation of germanium has recently been found in lignite remains of *Cupressinoxylon wardi* Knowlton, 1889, from the Patuxent formation (Lower Cretaceous) in the District of Columbia and vicinity. The discovery was made in the course of spectrographic studies on concentrations of germanium, gallium, vanadium, and other elements in ash of American coals.

The highest content of germanium heretofore reported was in germanite from Tsumeb, Southwest Africa, and from the Belgian Congo; that mineral contained 6–10 percent. The ash of *C. wardi* contains up to 6 percent and many of the samples contain 3–5 percent. The ash content of the samples (air-dry basis) is between 2 and 9 percent. The average content of germanium in the crust of the earth is estimated to be 1×10^{-4} percent. Consequently, in the ash of *C. wardi* the concentration is more than 10,000 times the average.

The ashes of samples of *C. wardi* also contain vanadium (0.7%–5.0%), chromium (0.1%–0.8%), and gallium (0.03%–0.2%). Some of the samples show a large concentration of copper. Examination of the ash of Pleistocene wood in this area indicates contents of a few hundredths of 1 percent of germanium.

The *C. wardi* was identified by R. W. Brown of the U. S. Geological Survey. We are also indebted to Henry Mela, Jr., and F. S. Grimaldi of the Trace Elements Section of the Survey for confirmatory chemical analyses for germanium.

[1] Published by the permission of the director, U. S. Geological Survey.

Rapid Carbon Dioxide Test for Sickling

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Sickle cell disease is a notorious mimic and deceiver. There is need for a method to demonstrate sickling which is rapid, dependable, and simple. Sickling tests in present use are not completely satisfactory. The sealed moist preparation method (*5*), the moist stasis test (*9*), and the oil-sealed tube technique (*1*) may not give positive results for many hours. The rapid bacteriologic method (*11*) requires caring for the perpetuation of the culture and does not lend itself to use in the physician's office. Recently advocated methods involving the use of various reducing substances such as BAL (*12*), cysteine (*12*), hydrogen sulfide (*12*), sodium dithionate (*8*), sodium hydrosulfite (*4*), cevalin (*3*), and sodium bisulfite and ascorbic acid (*3*) share the common disadvantage that these reducing agents are extremely unstable and must be freshly prepared.

We wish, therefore, to describe a simple and rapid method, utilizing carbon dioxide, by which sickling can be regularly demonstrated within 5 min from the time the blood sample is drawn. The test can be conveniently performed in the physician's office.

Five to 10 ml of venous blood is collected in an oxalated tube. The blood is transferred to a 250-ml Erlenmeyer flask, and a stream of pure carbon dioxide from a small, commercially available cylinder is directed into the neck of the flask for 10–15 sec. The flask is then immediately stoppered, and the blood is gently swirled about several times. The blood darkens. After the flask has remained stoppered for 5 min, the cork is removed and a drop of blood is quickly transferred by means of a pipette to a clean cover glass. A vaseline-sealed preparation is immediately made on a slide, and the cells are viewed under high-dry magnification. Speed is essential in the transfer of the drop of blood from the flask and in making the vaseline-rimmed preparation. The presence of unequivocal sickling under high-dry magnification indicates a positive result.

Twenty-seven Negro patients who showed delayed sickling after 1–57 hr on the routine sealed preparations (*5*, *9*) all gave positive findings immediately with the carbon dioxide test. Ten Negro patients who showed no sickling on the standard sealed tests gave negative results with the carbon dioxide method; the negative findings in this latter group indicate that the procedure of the new test does not of itself cause sickling.

The number of susceptible cells that sickled under the conditions of the carbon dioxide method varied somewhat from case to case. In most instances it was estimated that a minimum of from 20% to 30% sickle cells was present, but in two of our positive cases lesser numbers were found. When present, sickling was always obvious and unequivocal. In all positive cases a conspicuous number of erythrocytes that did not sickle showed distortion and angularity, which was never seen in the negative controls and which evidently represents a

presickle stage. Rouleaux formation was uniformly absent in those cases showing sickling.

It has been established that sickling of susceptible erythrocytes will take place only when the hemoglobin in the cells is in the reduced state (*6, 7, 10*), and the reduction of hemoglobin, however brought about, is the basis underlying all tests for sickling. In the commonly used sealed preparations (*1, 5, 9*), the metabolism of the nucleated blood cells slowly effects the reduction and produces sickling. In the bacteriological test (*11*) the high metabolism of the bacteria brings about more rapid reduction. The use of such agents as BAL, ascorbic acid, etc., depends upon their active reducing properties.

Carbon dioxide, in the technique described, plays a dual role in bringing about hemoglobin reduction. The gas displaces a large part of the air in the flask, thus producing a relatively oxygen-poor environment. In addition, the acid effect of the carbonic acid formed by the reaction of the gas with the blood decreases the affinity of the hemoglobin for oxygen (*2*).

There is little doubt that partial reoxygenation occurs during the transfer of the drop of blood from the flask to the slide. The percentage of cells that are sickled in the final preparation is, therefore, probably less than in the "flask blood" before transfer. It is, of course, possible to prevent this change by fixation of the cells in the flask by the addition of a saline formalin solution before taking a drop of blood for the preparation of the slide. This refinement was not felt to be needed in this test, however, because sickling is easily demonstrated without this step and because a qualitative rather than a quantitative result is all that is necessary.

The rapidity with which sickling can be demonstrated with the carbon dioxide test makes this method of great use in establishing an immediate diagnosis of sickle cell disease or in ruling it out, particularly in emergency situations. A negative reaction conclusively eliminates the possibility of sickle cell disease, whereas a positive reaction indicates that sickle cell disease is to be considered in the differential diagnosis. The simplicity of the test makes the procedure routinely practicable.

The dependability of the test is demonstrated by the absence of false positive and false negative reactions in the series of cases studied.

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To What Extent Is Oxygen Uptake of the Intact Embryo Related to That of Its Homogenate?[1]

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Recently, much attention has been given to analyses of the functions of constituent parts of living tissues, using homogenate and centrifuge techniques. That minute parts of cells can be isolated and concentrated rather easily was demonstrated clearly by Bensley and Hoerr (*1*). Further improvements in techniques and especially the use of high speed centrifugation have made possible detailed quantitative studies on the composition and functions of constituent parts of the living cell (*3–5*). Most of these investigations, however, have dealt largely with vertebrate tissues, and few if any seem to have been concerned with intact organisms and their homogenates. Considerable data are now available on the developmental history of the embryo of the grasshopper *Melanoplus differentialis*. It seemed of interest, therefore, to use such invertebrate material in further studying functions of cell constituents by homogenate and centrifuge techniques (*2*). Many features of this material make it especially desirable for such work. The embryos, free from yolk and in all stages of development, are easily obtained in large numbers (*2*). The egg is of a cleidoic type and hence is quite independent of the external environment for its food supply; it develops quite readily at room temperatures (25° C). Individual embryos, as well as morphologically and physiologically similar ones, are readily obtained, and from them homogenates are easily made. During the course of its development at 25° C, the embryo goes into a mitotically blocked or diapause state in which metabolic and other cellular activities reach a true basal rate (*2*). After removal of this developmental block, mitosis and other cellular activities are again resumed. A study has been made of the oxygen uptake of embryos, both intact and homogenized, and the results show rather striking properties of this material, as well as some differences from vertebrate tissues similarly treated.

Embryos of known age and temperature history were dissected free of yolk, as previously pointed out (*2*). A phosphate buffered Ringer's solution (pH 6.8) was used as suspension medium. Oxygen determinations were carried out at 25° C in Warburg manometers, using respiration flasks of 5-ml capacity. Intact embryos (100), as well as homogenates made from others of the same group, were run simultaneously. A glass type of homogenizer, as described by Potter and Elvehjem (*6*), and powered by an electric motor, was employed.

[1] Aided by a grant from the National Institutes of Health. Acknowledgment is gratefully made to K. H. Lu and Etta Andrews for technical assistance in carrying out these experiments.

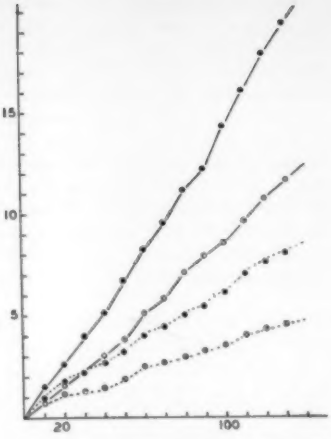

FIG. 1. Oxygen uptake of active and blocked intact embryos and homogenates; ordinates, mm³ O_2/100 embryos or equivalent; abscissas, time in min. Solid circles represent active embryos; open circles, blocked embryos; solid lines, intact embryos; broken lines, homogenates.

Results from many experiments seem similar, and in Fig. 1 are shown graphically typical curves for morphologically similar embryos and homogenates made from them. During diapause or block, oxygen uptake is always much lower than for active, nonblocked embryos. As a matter of fact, one judges the degree of block by the extent to which oxygen uptake is lowered in comparison with that of actively developing embryos. An inspection of Fig. 1 shows that oxygen uptake of intact embryos is always higher than that of the homogenates made from them. Oxygen uptake of homogenates for blocked embryos is correspondingly lower than that for homogenates of the active ones. The relative difference in oxygen uptake of embryos and homogenates, both for the active and blocked conditions, is surprisingly constant and has been found consistent in all experiments thus far carried out. Approximately 65% of the total respiration in both active and blocked cells is due to what one might term "physical structure or intactness," whereas approximately 35% is due to the basic chemical components of the system. A striking point is that a more or less linear relation between oxygen uptake and time for both intact embryos and homogenates is found (see Fig. 1). Dilution of the homogenates up to twenty times gives a straight line over a 2-hr period, showing that oxygen uptake is proportional to cellular concentration (Fig. 2).

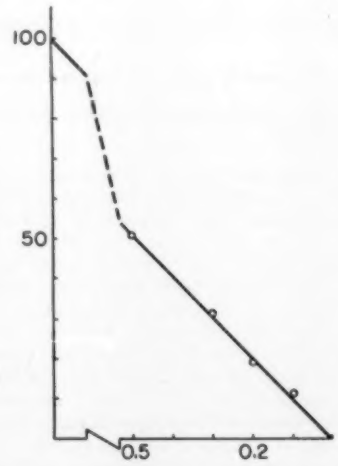

FIG. 2. Effect of dilution on oxygen uptake rate of homogenates, using original concentration (100 embryos/ml) as 100%; ordinates, % of normal oxygen uptake rate; abscissas, dilution of original homogenate.

Inasmuch as homogenates of active and blocked embryos show characteristic oxygen uptake, such systems when further analyzed should show physicochemical differences in the two physiological states of the cell. Further data on these points will be presented elsewhere.

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Effect of Zinc Deficiency on the Synthesis of Tryptophan by *Neurospora* Extracts[1]

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It has been reported that zinc is required directly for the synthesis of tryptophan and indirectly for the synthesis of auxins in tomato plants (*2*, *5*). It has also been demonstrated that tryptophan can be synthesized from indole and serine by wild-type *Neurospora* (*4*), as well as by cell-free extracts of the same organism (*6*). The present study with *Neurospora* indicates that cell-free extracts of zinc-deficient mycelia affect unfavorably the formation of tryptophan from indole and serine.

Cell-free enzyme extracts were prepared from the mats of *Neurospora crassa* (5297A) grown for 5 days in Fries basal medium, and from those grown on the same medium lacking zinc. The growth of the latter was ¼–½ that of the controls. The mycelial mats were washed with triple-distilled water, frozen, homogenized in three times their weight of 0.1M phosphate buffer at pH 7.5 and centrifuged. Five-tenths ml of the supernatant (10 mg dry

[1] Contribution No. 2 of The McCollum-Pratt Institute.

weight, corrected for buffer) was made up to 1.0 ml with additions of 102 μg indole (0.87 micromoles) and 2 mg DL-serine, and incubated at 37° C. Good agreement was obtained between indole disappearance and tryptophan formation utilizing Ehrlich's reagent essentially as described (*3*, *7*).

The considerably lessened activity of the zinc-deficient enzyme preparations could not be obtained with separate deficiencies of iron or manganese (Table 1). Lack of inhibitory effects resulting from intermixing experiments employing zinc-deficient and control extracts ruled out the possibility of the presence of an inhibitor in the zinc-deficient preparations. The addition of Zn^{++} (ranging from 2×10^{-6}M to 5×10^{-3}M in final concentrations) to the cell-free extracts of zinc-deficient material failed to restore enzyme activity. At concentrations of 2×10^{-4}M and 10^{-3}M, Zn^{++} actually caused a 66% and a 97% inhibition, respectively, in the controls. Exposure of zinc-deficient mats under sterile conditions to Zn^{++} at a level of 2 μg/ml (as in Fries basal medium) in a nitrogen-free medium did not enhance enzyme activity after 53 hr. Pyridoxal phosphate,[2] which has been shown to be active in this particular enzyme system (*6*), only slightly improved the synthesizing power of the zinc-deficient extract. Separate additions of Cu^{++}, Mg^{++}, Mn^{++}, and acid-hydrolyzed casein to the zinc-deficient extracts failed to restore enzyme activity. Cu^{++} was inhibitory to the control extracts at low concentrations, and Mg^{++} showed toxicity at higher values. Cysteine was ineffective in reactivating the zinc-deficient preparations and, at a final concentration of 3×10^{-2}M, caused a 59% inhibition in the activity of the control preparations. The metal binding agents, 8-hydroxy quinoline and ethylenediamine tetraacetic acid, proved to be ineffective in the control extracts as inhibitors except at high concentrations (0.3% and 2%, respectively). Potassium cyanide at final concentrations of 10^{-2}M and 10^{-1}M caused a 46% and a 92% inhibition, respectively, in the control preparations. Potassium thiocyanate gave 83% inhibition at 10^{-1}M.

TABLE 1

TRYPTOPHAN SYNTHESIS FROM INDOLE AND SERINE BY CELL-FREE ENZYME EXTRACTS FROM 5-DAY-OLD *Neurospora crassa* GROWN ON CONTROL MEDIA AND MICRONUTRIENT ELEMENT-DEFICIENT MEDIA

Cell-free extract	Indole lost	Tryptophan formed	Indole lost	Tryptophan formed	Indole lost	Tryptophan formed
	after 1 hr micromoles × 10^2		after 2 hr micromoles × 10^2		after 3 hr micromoles × 10^2	
Control mats						
Exp. 1	43	44	83	82	83	83
" 2	39	49	84	92	91	88
" 3	51	53	88	79	—	—
" 4 (3-day-old mats)	40	40	81	79	90	83
Average	43	47	84	83	88	85
Zinc-deficient mats						
Exp. 1	3	8	8	10	18	19
" 2	6	10	16	19	22	24
" 3	0	5	0	2	—	—
" 4 (3-day-old mats)	19	16	34	27	32	35
Average	7	10	15	15	24	26
Iron-deficient mats	31	35	65	66	84	94
Manganese-deficient mats	32	28	76	76	85	82

It would appear from these data that a relationship exists between zinc and the enzyme which converts indole and serine to tryptophan. It remains to be determined whether zinc is an actual constituent of the tryptophan-forming enzyme or whether it is concerned, directly or indirectly, in the synthesis of one or more constituents of the enzyme system.

Recent experiments in this laboratory also indicate that zinc deficiency leads to alterations in the activity (either increase or decrease) of certain enzymes, whereas other enzymes are not affected (*1*).

[2] Kindly supplied by I. C. Gunsalus.

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Vitamin P Protection against Radiation[1]

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Griffith *et al.* were the first to demonstrate the protective action of flavonoids in radiation injury (*2*). Clark and associates found that "a flavonoid preparation derived from lemons, administered in the drinking water to guinea pigs, reduces the mortality from total-body roentgen irradiation by about half" (*1*). Field and Rekers conducted an extensive investigation on the protective action of vitamin P factors, using dogs. Mortality after radiation was reduced to 10%–17% (with a significant reduction in the hemorrhagic diathesis) as against 60% mortality in the control animals. The investigators concluded "that previous misunderstanding of the nature of vitamin P has arisen from both the failure to recognize that several flavonone analogues pos-

[1] Condensation of paper presented at the annual meeting of the Florida Academy of Sciences, December 3, 1949, at Stetson University.

[2] Carll Tucker Fellow.

TABLE 1

CONTROL GROUP OF 20 IRRADIATED RATS*

No. of days of survival	11	12	13	14	15	16	17	18	19	20	21	22	23
No. of rats that succumbed	1	2	2	1	3	0	3	2	1	0	0	0	1

* These rats did not receive vitamin P.

sess very similar antihemorrhagic activity and that ascorbic acid has the capacity to potentiate activity in other flavonones'' (*3*, *4*).

In our investigation, 50 rats of British brown breed (obtained from Francis Carter Wood of St. Lukes Hospital, New York City) were submitted to x-ray irradiation. One group of 20 rats served as control, and a second group of rats was given vitamin P compound (CVP compound) isolated from citrus waste.[3] The average weight of the rats was 180 g, ranging from 160 to 205 g. The rats were kept on regular Purina Rat Ration. The radiation factors were 250 kv, 15 ma, with 0.5-mm Cu and 3.0-mm Bakelite filters. Target distance was 27.5 cm, and 210 r/min was the dose rate. All rats received 800 r total-body radiation in a single exposure.

Sixteen rats of the control group (80%) succumbed during the second and third weeks after the exposure (Table 1). All of them manifested gross hemorrhages of various gravity and pronounced pathological lesions in the adrenal glands. The zona fasciculata and zona reticularis were particularly affected, with argentaffin fibrils showing signs of degeneration. Four rats (20%) survived in spite of numerous petechial hemorrhages and generalized purpura.

TABLE 2

IRRADIATED RATS GIVEN 40 MG OF VITAMIN P

No. of days of survival	18	19	20	21	22	23	24
No. of rats that succumbed	1	0	0	0	1	1	1

The treated animals were divided into two groups. Ten rats received orally 4 mg of vitamin P compound per day for 10 days, 3 days prior to radiation and 7 days after radiation. Twenty rats received 5 mg of vitamin P per day for 30 days, 7 days prior to radiation and 23 days after radiation.

In the group of animals (Table 2) which received a total amount of 40 mg of vitamin P compound, the mortality from irradiation was reduced to 40%. Moreover, those rats which did not succumb to the injurious effect of radiation lived longer. The petechial hemorrhages in the treated animals were considerably less pronounced, but some pathological changes in the adrenal cortex were observed, mostly in the zona reticularis (vacuolization).

In the group given a total of 150 mg of vitamin P compound in a period of 30 days, mortality from irridiation was reduced to 10% (Table 3). In this group, petechial hemorrhages were very slight and in some rats apparently absent.

[3] This compound, containing four identified factors naturally present in citrus fruit, was obtained from Vitamerican Company, Paterson, New Jersey.

TABLE 3

IRRADIATED RATS GIVEN 150 MG OF VITAMIN P

No. of days of survival	18	19	20	21	22	23	24	25
No. of rats that succumbed	1	0	0	0	0	0	0	1

From these observations it appears that the vitamin P compound, which contains four flavonoids naturally present in citrus fruit, gives considerable protection to rats against a total-body, near-lethal dose of radiation.

In our previous publication (*5*), we stressed the importance of making a clear distinction between increased capillary permeability and capillary fragility. In radiation injury, there seems to be present a pronounced increase in capillary fragility which might be prevented by large doses of flavonoids naturally present in citrus fruit.

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Likelihood of Photorespiration or Light-inhibited Respiration in Green Plants[1]

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Generally speaking, there are two ways in which light could stimulate respiration: either directly by photosensitization or indirectly through photosynthesis, thereby increasing the amount of respirable metabolites. It is the direct effect of light on respiration (photorespiration), commencing upon exposure to light and stopping immediately upon the return to darkness, that has been a source of disturbance to those interested in measuring photosynthesis, as such a process escapes measurement. Whenever light has been reported to stimulate respiration, the stimulation has been of the persistent or indirect type and could be explained either on the basis of an accumulation of photosynthates, or on the basis of light absorption by the carotenoids, in which case respiration has been found to increase slowly in the light and persist for a short time in the dark. The possibility also

[1] This experiment was part of a dissertation presented in 1949 in partial fulfillment of the degree of Doctor of Philosophy in Yale University.

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exists that light inhibits respiration. It would follow that photosynthetic determinations above and also below the compensation point, where oxygen evolution is measured as a decrease in respiration, would become of doubtful significance.

McAlister (*8*) concluded from his experiments with wheat seedlings that light does not have a direct effect on respiration, since the rate of respiration after a period of illumination was equal to that before illumination and was independent of light intensity. Gaffron (*6*) arrived at a similar conclusion from an experiment in which cyanide inhibited the respiration of *Scenedesmus* by approximately 96% but had no effect on the calculated "true photosynthesis." Such a result can also be interpreted on the basis that cyanide does not affect photorespiration. The fact that Emerson and Lewis (*4*) did not find an effect of temperature on the calculated quantum yield also suggests that light does not have a direct effect on respiration. More recently, Burk *et al.* (*2*) and Warburg *et al.* (*10*) came to a similar conclusion. Kok (*7*) concluded, from experiments in which oxygen evolution was measured in the dark and in light of increasing intensity, that respiration is inhibited at high light intensities.

All such experiments, however, are of an indirect nature and have, therefore, the limitations inherent in indirect experimentation. Although results obtained thereby are frequently correct, they should, if at all possible, be tested with a direct method. Heretofore, the separation of photosynthesis from respiration in light was only possible by the use of poisons, which in many instances are undesirable because of their known or unknown effects on processes other than those to be specifically inhibited. A technique is now available by which these difficulties can be eliminated. In artificially induced mutant strains which are unable to photosynthesize or evolve oxygen in light, but yet are green, respire, and grow when supplied with a suitable carbon source, photosynthesis is completely divorced from respiration, and the problem of whether or not light directly affects respiration is rendered amenable to direct experimentation. Such mutant strains of *Chlorella* have been obtained as the result of ultraviolet irradiation and have been the object of photosynthetic studies (*3*). Strains 322 and 349 which possess these characteristics afforded an excellent opportunity for the study of the influence of light on respiration. If a process such as photorespiration, or light-inhibited respiration, exists, it would be extremely likely that it could be demonstrated by determining the respiration rates of these strains in the light and in the dark.

Oxygen-uptake measurements were made with the cells suspended in 0.1M Emerson and Lewis carbonate bicarbonate buffer solution (*5*), as these experiments were performed in conjunction with oxygen liberation studies. The cells were grown on solid stock medium slants which contained the following, per liter of nutrient solution: KNO_3, 1.21 g; $MgSO_4$, 1.20 g; KH_2PO_4, 1.22 g; 1,000 × Pratt's iron solution (*9*), 1 ml; Arnon's A4 trace element solution (*1*), 1 ml; tryptone, 4.5 g; dextrose, 10 g; yeast extract, 0.36 g; agar, 10 g; distilled water, to make 1 liter. Cultures were grown under continuous light for 11 days, after which the cells were harvested, washed in distilled water, and suspended in 0.1M carbonate bicarbonate buffer solution. Two-milliliter portions of the suspensions were added to the main chambers of Warburg reaction vessels. The vessels were attached to manometers, immersed in a constant temperature water bath (27° C), and shaken. After 15 min for equilibration, readings were taken during 30-min light periods and 30-min dark periods. The light intensity at the level of the vessels was about 500 ft-c. Wet-packed cell volumes were determined from aliquots of the suspensions.

TABLE 1

RESPIRATION RATES OF MUTANT STRAINS OF *Chlorella* IN LIGHT AND IN THE DARK

Strain	Q_{O_2} (cu mm of O_2/hr/cu mm of cells)	
	In dark	In light
No. 322	−1.64	−1.77
	−1.76	−1.70
	−1.70	−1.39
	−1.52	−1.20
	−1.64	−1.26
	−1.26	−0.95
	−2.13	−1.73
	−1.79	−0.98
	−1.90	−1.84
	−1.33	−1.44
	Average = −1.67	Average = −1.43
No. 349	−3.98	−3.85
	−3.79	−4.05
	−3.66	−3.92
	−3.34	−3.34
	−3.39	−3.51
	−3.62	−3.34
	−3.45	−3.63
	−3.82	−4.01
	−3.95	−3.95
	Average = −3.67	Average = −3.73

Respiration rates (cu mm of oxygen taken up per hr per cu mm of cells), calculated from the amounts of oxygen taken up during the 30-min light and dark periods, were determined for several cultures of each strain. The results indicate that light had no effect on the respiration of strains 322 and 349, as their respiration rates were approximately equal in the light and in the dark (Table 1). It is noted that positive pressure changes were not observed upon exposing these strains to light, as is normal and as is found for the parental type. The argument could be presented that the gene mutations which resulted in the inability of these strains to photosynthesize also resulted in their inability to carry on photorespiration. This is unlikely, however, as there is considerable evidence that indicates they are blocked in different reactions, and neither exhibited a significant increase in respiration in the light.

This experiment presented an excellent chance for photorespiration to be exhibited if it existed. However, it was not observed. Also, since the rates of respiration were approximately the same in the light and in the dark, it is extremely unlikely that light inhibits respiration. It seems certain, therefore, that light does not have a direct effect on respiration.

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Enzymic Conversion of Maltose into Unfermentable Carbohydrate

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Pigman (*6*) and Stark (*9*) reported that amylase preparations from molds, pancreas, and saliva are capable of synthesizing unfermentable carbohydrate or carbohydrates from maltose. However, the work of these authors is incomplete, since they did not separate or characterize the synthesized product.

While studying the enzymic hydrolysis of starch in distillery corn mash, by the use of submerged fungal cultures, we found that the secondary conversion of dextrins[1] into fermentable sugars was markedly inhibited by maltose.[2] The question was therefore raised as to whether the observed reduction in the rate of dextrin hydrolysis was actually a result of maltose inhibition or of the conversion of maltose into unfermentable carbohydrate. Conclusive data have since been obtained to show that such an enzymic conversion actually occurs.

The submerged fungal culture was prepared with *Aspergillus niger* NRRL 337 in a distillers' dried solubles-corn meal medium (*1*). One volume of the culture filtrate was mixed with two volumes of a 2.2% maltose[3] solution in 0.3 M acetate buffer (pH 4.4) and incubated at 30° C. Samples were taken periodically and analyzed for glucose, maltose, and unfermentable carbohydrate (dextrins) according to the tripartite method[4] of Stark and Somogyi (*10*). The data given in Fig. 1 show that, while a part of the maltose was hydrolyzed to glucose through maltase activity, another part was simultaneously converted into unfermentable carbohydrate. The presence of unfermentable material at zero hour was ascribed to dextrins contained in the fungal culture and in the maltose. During the reaction period the amount of unfermentable substance increased considerably, reaching a maximum at 8 hr, when 17.6% of the original maltose had been converted to unfermentable carbohydrate. A decrease in unfermentable material was observed after the maltose had been completely utilized (10–12 hr). The amount remaining accounted for only 10.2% of the maltose at the end of 24 hr. It is therefore concluded that the fungal culture must contain some enzyme or enzymes which catalyze the conversion of maltose into unfermentable carbohydrate. In the absence of maltose, this carbohydrate can be converted into glucose.

A similar experiment, in which glucose was used in place of maltose, showed no formation of unfermentable substance; the amount of glucose remained unchanged during the 24-hr reaction period. Apparently the enzyme or enzymes were inactive toward glucose.

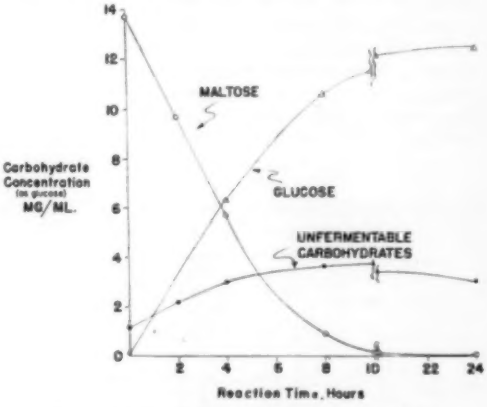

Fig. 1. Action of fungal enzymes upon maltose.

As a further confirmation of the formation of unfermentable substances, a similar experiment was conducted using a 10% maltose solution. Instead of using buffer, the filtrate of fungal culture was adjusted to pH 4.4 before mixing with the maltose solution. Ammonium sulfate, at a concentration of 1.5 g/l, was added as nutrient for subsequent yeast fermentation. After the fungal enzymes had been in contact with the maltose for 24 hr at 30° C, the mixture was sterilized and inoculated with an actively growing culture of distiller's yeast which had been grown in a yeast extract-glucose medium. At the end of fermentation (3 days), the medium was found to contain 40.7 mg of unfermentable carbohydrate per ml, equivalent to 41.5% of the maltose used (Table 1). The same table shows that the reaction mixture, which was sterilized immediately after mixing the maltose solution and the filtrate of the fungal culture, con-

[1] Like barley malt, the fungal culture enzymes hydrolyze starch primarily into fermentable sugars and dextrins.

[2] A report including this finding has been prepared for publication.

[3] Maltose (cp Fisher) recrystallized in the laboratory.

[4] Carbohydrate contents of samples either before or after treatment with baker's yeast were all determined as glucose by Somogyi's method (*8*) after complete acid hydrolysis in 0.69 *N* HCl solution for 2.5 hr in boiling water.

tained no such product; neither did one in which glucose was used in place of maltose.

TABLE 1

ENZYMIC CONVERSION OF MALTOSE TO UNFERMENTABLE CARBOHYDRATES

Glucose used in reaction mixture mg/ml	Maltose used in reaction mixture mg/ml	Reaction period total hr	Unfermentable carbohydrates after yeast fermentation mg/ml
97.0	...	0	2.72
97.0	...	24	3.04
...	98.1	0	3.38
...	98.1	24	40.7

In order to obtain data on the nature of the unfermentable carbohydrate, it was separated from the fermented medium by the following procedure. Extraneous material was removed from the fermented medium by precipitation with barium hydroxide, followed by ammonium carbonate. The filtrate was evaporated to dryness on a steam bath, and the residue taken up in a small amount of water. The addition of methanol resulted in a precipitate which had a carbohydrate content equivalent to 5% of the total carbohydrate in the original fermented medium. Acetone, added to the methanol solution, produced a brown, syrupy precipitate. This precipitate, after washing with acetone and drying under vacuum, analyzed 88.5% carbohydrate (as glucose, after acid hydrolysis), equivalent to 74.8% of the unfermentable carbohydrates in the original fermented medium. This was considered the crude product of the unfermentable carbohydrate produced enzymatically from maltose.

The crude product was purified further after dissolving in water to make a 4% solution. This solution was alternately treated with ion-exchange resins Amberlite IR-100 and IR-4B, and the resulting solution was then completely decolorized with Darco activated carbon. The solution was then evaporated to a thick syrup. Traces of insoluble solids were removed from the syrup by centrifugation. The addition of five volumes of methanol to the syrup produced a small quantity of precipitate with a carbohydrate content equivalent to 4% of the total amount present. The methanol solution was poured into ten times that volume of acetone, and a white, curdy precipitate was obtained. This precipitate was filtered with suction, dried overnight at 56° C under 28–29 in. Hg vacuum, and kept in a desiccator. The dried product was highly hygroscopic and analyzed 92.8% carbohydrate. A total of 4.5 g of this product was obtained from 9.5 g of the crude product.

TABLE 2

REDUCING POWERS OF THE UNFERMENTABLE CARBOHYDRATES

Samples	Glucose content* after acid hydrolysis (A)	Glucose content calculated from direct reducing power: Toward Cu-tartrate-phosphate reagent* (B)	Glucose content calculated from direct reducing power: Toward NaIO† (C)	Ratio of direct reducing power to total reducing power after acid hydrolysis: (B) : (A)	Ratio of direct reducing power to total reducing power after acid hydrolysis: (C) : (A)
	%	%	%		
Original fermented medium	4.07	1.71	...	1 : 2.38	.....
Crude product	88.5	35.4	36.3	1 : 2.49	1 : 2.43
Purified product	92.8	38.9	33.5	1 : 2.38	1 : 2.76
Maltose monohydrate	99.5	55.7	48.7	1 : 1.78	1 : 2.04

* Determined from reducing power toward Somogyi's Cu-tartrate-phosphate reagent.

† By the method of Caldwell *et al.* (2), which was found to be less sensitive toward impurities contained in the fungal culture than the original method of Willstätter and Schudel (Green [3]).

TABLE 3

RECONVERSION OF UNFERMENTABLE CARBOHYDRATES TO FERMENTABLE SUGARS BY FUNGAL ENZYMES

Carbohydrate substrate	Yeast inoculum added	Fungal culture added % (by vol)	Initial carbohydrates mg/ml	Unfermentable carbohydrates after 3-day incubation mg/ml
Crude product	+	10	20.5	4.0
Crude product	+	0	22.0	21.4
Purified product	+	10	10.2	3.2
Purified product	+	0	10.2	10.1

Reducing powers (calculated as glucose), determined before and after acid hydrolysis of the unfermentable carbohydrate, are listed in Table 2. For comparison, the reducing powers of the recrystallized maltose monohydrate are also given. The last two columns give the ratios of these reducing powers—an indication of the number of glucose units per molecule or chain length (*4*). The values in these columns show that the unfermentable carbohydrate must have an average molecular size somewhat greater than maltose, but less than three glucose units per molecule.

Fermentability of the crude as well as the purified product was tested by inoculating media containing these two products (plus 1.5% yeast extract) with distiller's yeast. Little or no yeast growth and no diminution of the carbohydrate content were observed after 3 days' incubation at 30° C. When 10% (by volume) of submerged fungal culture and yeast was added to these media, fermentation (gas evolution) took place and the carbohydrate disappeared. These data, which are summarized in Table 3, show that the product can be recon-

verted into fermentable sugars if they are continuously removed by yeast fermentation.

The data reported here confirm the report by Pigman (*6*) that a mold enzyme or enzymes can convert maltose to some unfermentable carbohydrate or carbohydrates. The possible occurrence of such enzyme or enzymes in amylolytic preparations undoubtedly explains the apparent inhibition of amylase activity by maltose as reported by Schwimmer (*7*), and the reversibility of enzymic hydrolysis of dextrins as observed by Stark (*9*). The small molecular size of this unfermentable carbohydrate suggests that it might consist partly or entirely of isomaltose (6-[α-D-glucopyranosyl]-D-glucose), which has been isolated by Montgomery *et al.* (*5*) as one of the end products of prolonged starch hydrolysis by taka-amylase, although these authors obtained no isomaltose from maltose. Studies to elucidate the chemical nature of the product, as well as details of the enzymic reaction, are in progress.

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Activation of Arginase *in Vitro* by Mouse Carcass Extract and the Cobalt Ion

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While pursuing another phase of work on the enzyme arginase, it was observed that there might be a sex-linked difference in tissue activity in addition to general tissue variables observed by Greenstein (*1*). Further, it appeared possible that a sex-linked difference could be due to the presence in tissue of an activator other than cobalt.

In this study, the arginase activity was determined according to the method of Mohamed and Greenberg (*3*), using cobalt as metal activator, at a temperature of 40° C, for a period of 30 min. All values are the result of triplicate determinations. As seen in Table 1, the average value for 9 standard preparations of young female bovine livers, with cobalt as the activator, amounts to 377 arginase units; the average of 6 young castrated male bovine livers (cobalt-activated) amounts to 242 arginase units.

TABLE 1

Type of enzyme preparation	Type of activator	Avg arginase units* (*1*) (±2)	Percentage original units†
Young ♀ bovine liver	Co^{++}	377	100
	♂ extract	193	51
	♀ extract	168	45
	none	35	9
Young castrated ♂ bovine liver	Co^{++}	242	100
	♂ exract	176	73
	♀ extract	113	47
	none	35	14

* Per unit of standard preparation. Nine ♀ bovine livers and 6 ♂ bovine livers were used to obtain these averages.
† Ratios for both enzyme preparations with respect to each type of activator.

It appeared possible that the difference noted might be sex-linked. To test this possibility, arginase activity was determined on each of the skinned and eviscerated carcasses of 20 female and 20 male Swiss strain mice. Approximately 1 g of each mouse carcass was ground in a Waring Blendor with 2.5 ml of pH 7.0 phosphate buffer. In Table 2 there is observed approximately a fourfold difference between male and female mouse carcasses in arginase activity when cobalt is used as an activator. In the absence of cobalt there is practically no activity and no difference between male and female mouse carcasses.

In Table 1 it can be seen that a mixture of mouse carcass extract (male or female) and bovine liver arginase, in the absence of added cobalt, shows a value many times higher than either tissue preparation alone. A substance or substances in one of the tissue preparations (liver or carcass) serve as an activator for the enzyme in the other preparation. Since the standard liver preparations represent a partially purified solution

TABLE 2

Type of enzyme preparation	Type of activator	Avg* arginase units per gram of mouse carcass (±2)
♂ Mouse extract	Co^{++}	330
♀ Mouse extract	Co^{++}	90
♂ Mouse extract	none	11
♀ Mouse extract	none	12

* Average computed from determinations made on 20 ♂ and 20 ♀ Swiss-strain mice.

of the enzyme, it seems reasonable, for the present, to attribute the role of activator to the unpurified carcass preparations. Why such a carcass activator does not activate the carcass arginase cannot be stated at this time.

It seems probable that there is present in tissue an activator (or deactivator of an inhibitor) of arginase that may control the rate or degree of activation *in vivo*. Perhaps such an activator may play a role in the control

of arginase activity in malignant tissue (*2*); possibly this activator is sex-linked.

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Further Study of the Role of Hyaluronidase in the Fertilization of Rabbit Ova *in Vivo*[1]

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In a previous experiment (*4*), superovulated doe rabbits were inseminated with 0.5 ml of a sperm suspension (1: 1,000 in 0.9% NaCl) added to 0.5 ml of 0.9% NaCl containing partially purified testis hyaluronidase. Although the number of fertilized ova was not increased by the addition of hyaluronidase, it was thought that the dilution effect (*3, 6*) may have reduced fertilizing capacity and that the partially purified hyaluronidase may have contained inhibitory impurities. Since hyaluronidase is thought to be of therapeutic value in oligospermia in human infertility (*8*), a further test of a possible hyaluronidase effect on the fertilization of rabbit ova *in vivo* was conducted.

Seminal plasma has been demonstrated to be beneficial to the fertilizing capacity (*4, 5*) and to the motility (*6*) of spermatozoa in the highly diluted form. Therefore, 0.003–0.01 ml of rabbit semen was suspended in a fluid containing 1 ml of vasectomized seminal plasma (which itself has no hyaluronidase activity) and 9 ml of Fructose Ringer's solution (*5*). In one group of animals, 1 mg of highly purified testis hyaluronidase[2] in 0.5 ml of Fructose Ringer's solution was placed into the upper part of the vagina. The animals were then inseminated with 1 ml of the sperm suspension. In the second group, rabbits were inseminated with 1 ml of the sperm suspension containing 1 mg of purified hyaluronidase. In a third group, the animals were inseminated with 1 ml of the same sperm suspension containing 1 mg of lyophilized, vasectomized rabbit seminal plasma. The number of spermatozoa in the sperm suspension was estimated with a hemocytometer. The animals were injected intravenously with sheep pituitary extract just after insemination in order to induce ovulation. They were sacrificed 25 or 72 hr later. The ova were recovered by flushing the tubes, and fertilized as well as unfertilized ova were counted.

Results are presented in Table 1. The average percentage of fertilized ova is 50 and 59 in the experimental groups, and 46 in the control group. The percentage of total fertilized ova is 42 and 55 in the experimental groups, and 39 in the control group. There is no significant difference between each group, either calculated according to the χ^2 test or according to the t test. It is not established, therefore, that the application of hyaluronidase in the vagina before insemination or the addition of hyaluronidase to the sperm suspension has any effect on the fertilization of rabbit ova *in vivo*.

TABLE 1

Effect of Purified Testis Hyaluronidase on Fertilization *in Vivo*

Experiment No.	No. of sperm inseminated	No. of ova*		% fertilized	No. of ova†		% fertilized	No. of ova‡		% fertilized
		Fertilized	Total		Fertilized	Total		Fertilized	Total	
1	250,000	8	34	24	22	26	85	9	32	28
2	210,000	7	15	47	10	10	100	21	22	96
					8	28	29			
3	150,000	24	27	89	7	10	70	15	17	88
4	50,000	8	12	67	38	55	69	8	47	17
5	80,000	2	10	20	5	11	46	6	22	27
6	93,000	1	58	2	4	30	13	2	41	5
7	300,000	27	27	100				29	50	58
Avg. % fertilized ova per doe				50			59			46
Total ova in each group		77	183	42	94	170	55	90	231	39

* Hyaluronidase (1 mg) placed into vagina before insemination.

† Hyaluronidase (1 mg) added to sperm suspension.

‡ Dried vasectomized semen (1 mg) added to sperm suspension.

The hyaluronidase used in the present experiment was tested by the viscosimetric method (*7*). It was estimated to be about 200 times as potent as the partially purified hyaluronidase used in a previous experiment (*4*). Vasectomized semen has no hyaluronidase activity

[1] This investigation was supported by a grant from the Committee on Human Reproduction, National Research Council, acting on behalf of the National Committee on Maternal Health. Thanks are due to Dr. G. Pincus for encouragement.

[2] Kindly supplied by Dr. Joseph Seifter, and containing 800 turbidometric units per mg.

(*4*), and the hyaluronidase activity of the sperm suspension employed in the present control group was practically nil. However, the percentage of fertilized ova in the control group and in the experimental group was very similar. The motility of spermatozoa in the sperm suspension with or without hyaluronidase was the same; all showed active motility in the highly diluted form at room temperature for 6 hr. The role of hyaluronidase as determined by the observation of dispersing cumulus cells *in vitro* does not appear to be so important in fertilization as previous investigators have thought because fertilized ova were still observed in cumulus cells (*1*, *2*, *9*). The experimental findings here reported fail to support the claims made for the clinical use of hyaluronidase.

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A Simple Staining Technique for Detecting Virus Diseases in Some Woody Plants[1]

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During the course of biochemical studies on virus diseases affecting deciduous fruit trees, it was observed that virus infection induces the accumulation of phenolic compounds in affected tissues. A color test for these compounds was developed (*2*), which proved useful in the study of various stone-fruit virus diseases.[2] Further work suggested that if these phenolic compounds could be fixed in the tissue and subsequently stained, they might serve as a means of observing virus distribution in the plant. Such a test was devised for the staining of phenolic compounds in cleared leaf tissue. The stained compounds are not distributed uniformly throughout the tissue but are located in patterns which are characteristic for the type of virus and for the severity of infection. The technique is useful in detecting and studying what appear to be virus movement and distribution in plants capable of producing polyphenols similar to those produced by deciduous fruit trees.

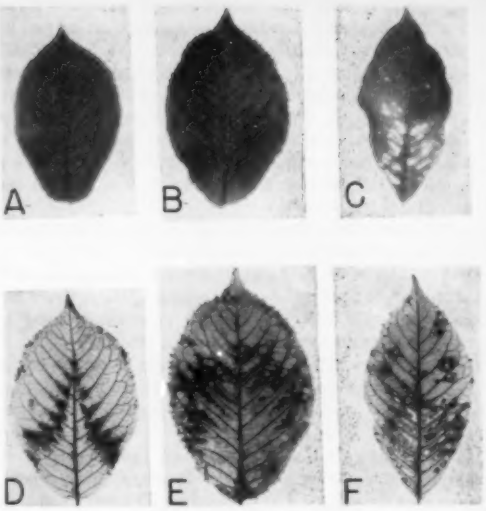

Fig. 1. Bing cherry leaves affected with ringspot. A, B, and C, before clearing and staining; D, E, and F, the same leaves after staining. The shot holes in C and F are caused by the virus.

The staining procedure consists of removing the chlorophyll, fixing the polyphenols, and developing blue-colored polyphenol compounds by treatment with NaOH. Whole leaves or sections of leaves were used. This procedure was also used with sections of petioles, stems, and roots. The decolorizing and fixing are performed in one operation by using a reagent composed of 700 ml of 95% ethyl alcohol, 20 ml of 37% formaldehyde, and 230 ml of distilled water. Samples may either be boiled in this solution under a condenser, or they may be heated in a water bath at 80° C until the chlorophyll is completely removed. When the clearing and fixing reagent becomes strongly colored, it should be discarded and fresh reagent added. One or two changes are usually sufficient. The chlorophyll from young leaves is removed in 5 to 10 min, but older leaves may take as long as 2 hr to clear. When decolorized, the leaves appear white or light yellow.

After decolorization, the samples are transferred to normal NaOH and heated at 80° C to 100° C until maximum color develops. The time of heating varies with the type and age of the sample but is usually from 2 to 10 min. A deep blue color indicates a test reaction between polyphenols and NaOH. Tissues in which these phenolic compounds are absent are yellow. The blue color of the phenolic compounds stands out sharply

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[2] A recent comment in *Science* (*1*), suggesting that our original color test for virus diseases was due to reducing substances, is in error. A small amount of copper sulfate was added to the reagent for the colorimetric test, to catalyze the oxidation of the polyphenols. The author of the comment assumed that a cuprous oxide precipitate was the basis for the test, whereas a red-colored solution was originally reported. Copper is not essential to color formation in this test.

against this yellow background, as may be seen in Fig. 1.

The blue color developed with this method is not permanent but oxidizes to a red color in 5 to 10 min when exposed to air. Acidification of the sample also changes the blue color to red. The red color persists for a month or more. Treated tissue may be placed in lactic acid after staining if it is to be kept for any length of time. The lactic acid not only changes the blue color to a more stable red, but at the same time makes the leaf more transparent.

The results with the whole-leaf staining procedure can be observed macroscopically or with the aid of a low power microscope, so that the usual histological techniques of embedding, sectioning, and staining were not employed. However, the method presumably could be adapted for more precise histological studies.

It should be noted that the procedure is not applicable to all plant species but is restricted to those which have a polyphenol system similar to that present in deciduous fruit trees. The following virus-infected plants were found to give good staining reactions: peaches, cherries, apricots, and plums (*Prunus*); apples (*Malus*); pears (*Pyrus*); strawberry (*Fragaria*); rose (*Rosa*); and high bush cranberry (*Viburnum*). Negative results were obtained with all the viruliferous herbaceous plants—other than the semiherbaceous strawberry—thus far tested.

Since this method is based upon the accumulation of certain polyphenols in virus-infected tissue, care should be taken in the sampling procedure and the interpretation of results, because conditions other than virus infection may induce accumulation of the same or similar phenolic compounds. Nectaries on the leaf normally stain very deeply. Any type of phloem blockage (girdling) may produce phenol accumulation. Similarly, mechanical injuries to the leaf often induce the accumulation of the phenolic compounds, as a wound reaction, immediately adjacent to the injury. Virus diseases, however, produce characteristic distribution patterns of the phenolic compounds and, therefore, can usually be distinguished from other more localized conditions.

The type of staining pattern produced in leaf tissue invaded by different viruses depends upon the kind of virus and the stage of development of the disease. In the case of western X-disease of peaches, a yellows-type virus disease, staining is confined to the phloem in the veins of the leaf during the early stages of the disease. As the disease progresses, localized areas of parenchyma tissue adjacent to the veins become involved, and finally most of the leaf stains deeply throughout. The ringspot diseases of cherries, either in latent or visible stages, produce striking staining patterns (Fig. 1). In the strictly latent stage the staining is confined to the phloem in the leaf veins. In those forms showing symptoms, even though so faint as to be nearly indiscernible, characteristic distribution patterns in the leaf parenchyma are produced that correspond to the symptom patterns.

Stem sections have not been as useful as those of leaves, because the nature of the tissue precludes the production of specific staining patterns. The staining is usually confined to the phloem, but xylem and phloem rays, pericyclic parenchyma, and sometimes cortical and pith parenchyma also will stain, depending upon the severity of the disease.

Genetic variegations may produce chlorophyll distribution patterns in a leaf that resemble virus disease symptoms. None of the genetic variegations tested has reacted to the stain test. Similarly, certain insecticides and other chemicals will induce viruslike symptoms in the leaf, and these also failed to give a stain reaction. The accumulation of polyphenols in virus-affected tissue is not completely understood, but apparently it is not related to the reactions involved in chlorophyll destruction. It seems likely that one or both of the following mechanisms is involved: (1) partial or complete blockage of the phloem, resulting in the dehydration of accumulated sugars to polyphenols; (2) mutual precipitation of virus protein and polyphenol, and by mass action a subsequent accumulation of polyphenol-virus aggregate. In the latter event, the staining reaction may be a test for the localization of inactivated virus particles.

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Biological Experiments on *Drosophila melanogaster* with Supersonic Vibrations

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Recently, supersonic waves have begun to be widely used by physicians to treat such diseases as rheumatism, ulcus cruris, etc. It is, therefore, necessary to make an exact examination of the biological effects of these vibrations. It is first of all important to know whether supersonic waves have any mutagenic influence on the genes.

Many biological experiments with supersonic vibrations (hereafter to be called S.V.) have been made, but mostly with the purpose of determining the killing effect against protozoa, bacteria, or small experimental animals such as mice. Hersh *et al.* (*2*), who made genetic experiments with *Drosophila melanogaster*, treated them in the air, not being aware that air cannot conduct sonic waves of high frequency. We have made some further genetic experiments by investigating the effects of S.V. on mutations and development in *Drosophila melanogaster*.

We used a wild stock of *Drosophila*, treating all stages of development, i.e., egg, larval, and pupal. The insects were placed in an isotonic NaCl solution in a tube of artificial resin, Trolitul, with a bottom of special thickness to let the waves through. This receiver was put on the vibrator head of a piece of equipment used by physicians (an ultravibrator) which has an intensity of 0.7–4 w/cm² (800,000 vibrations per sec). To exclude a heat effect, we cooled the receiver with water, thus allowing a treatment of over 25 min without a significant increase in the temperature of the NaCl solution.

TABLE 1

DIFFERENCE IN SUSCEPTIBILITY TO S.V. (INTENSITY 1.75 W/CM²) IN DIFFERENT STAGES OF DEVELOPMENT

Age in hr when treated	Stage	Time of exposure	Percentage of survivors after treatment	Percentage of imagoes	Modified organs
2	egg	5 sec	12.1	12.1	none
15	"	60 "	75.6	47.1	legs, wings, abd.*
22	larval I	10 "	57.3	51.2	eyes, hyp.*
42	" I	30 "	57.7	37.2	abd.
70	larval III	30 "	13.2	7.3	abd., wings
70	" III	60 "	8.3	4.2	none
100	larval III	15 "	87.3	60.7	hyp.
100	" III	30 "	82.4	28.9	hyp.
100	" III	60 "	63.9	11.8	hyp.
108	prepupal	60 "	53.8	25.6	hyp.
110	"	30 "	64.0	41.7	hyp., legs
113	"	60 "	81.8	44.2	hyp., legs, eyes
115	"	30 "	100.0	85.7	hyp., legs, eyes, wings
122	pupal	4 min	63.6	45.5	" " " "
126	"	5 "	100.0	96.2	" " " "
130	"	10 "	52.2	41.5	" " " "
130	"	20 "	33.3	25.0	" " " "
160	"	10 "	55.0	65.0	" " " "
180	"	10 "	100.0	100.0	wings
200	"	10 "	100.0	100.0	"

* Abd. = abdomen; hyp. = hypodermis.

We observed a big difference in susceptibility in the various stages of development (see Table 1). The eggs, larvae, and early pupae (prepupae) are extremely susceptible to S.V. Usually the insects survive after treatment, but some time afterwards a few of them die, while others die at a later stage of development. The metamorphosis, which begins with the formation of a puparium, seems to be a very sensitive one. After a pupation of about 7 hr, resistance begins to increase slowly, and 6 hr after metamorphosis is finished, resistance increases rapidly.

In 22-hr pupae exposed to S.V. for 20 min at an intensity of 1.75 w/cm², mortality was 75%. A treatment lasting 1 min, however, killed the same number of 1-hr pupae. This means that resistance increases quickly to approximately twentyfold what it is at the beginning of puparium formation, and to nearly a thousandfold as compared with the resistance of the egg when laid. In the other stages the same effect has been observed, i.e., resistance increases with age, and this more strikingly in the egg stage than in the larval. This result parallels the effects on *Drosophila* eggs with x-rays (*4*). It seems probable that morphological changes in prepupal or early egg stages of a growing insect are more susceptible to S.V. than are simple proliferations or cytological differentiations of tissues. A possible explanation of the great resistance of old pupae might be the existence of air, between pupa and puparium, that does not conduct S.V.,

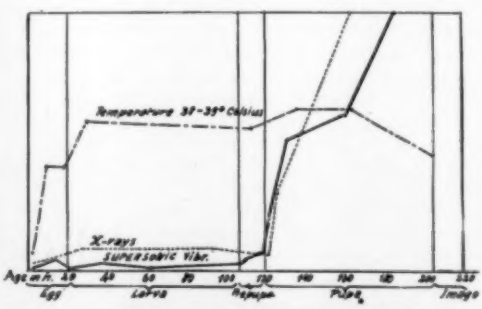

FIG. 1. Differences in susceptibility to different agents in various stages of development. Ordinates: for broken line (temperature 38°–39° C during the 2–3-hr period of the test)—% of survivors (Henke [*1*]); for dotted line (x-rays, dose to kill 50%)—0–2,400 r (Mavor [*3*]; for solid line (supersonic vibrations, 1.75 w/cm² for time needed to kill 50%)—0–20 min.

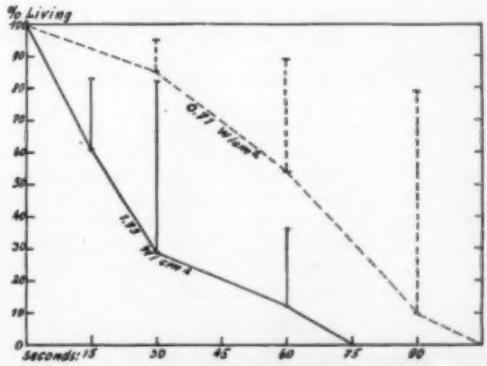

FIG. 2. Variation in survival rate of larvae (100 h. old) with time; treatment with 0.71 and 1.75 w/cm². Curve represents number of emerged imagoes. The percentage of survivors some time after treatment is plotted for each value.

TABLE 2

THE EFFECT OF SUPERSONIC WAVES ON MUTATION RATE

Stage when treated	Treatment	Number of tested X-chromosomes	Number of lethals
Egg	60 sec 1.75 w/cm²	93	..
Larval	0.71 and 1.75 w/cm², 10–60 sec	79	..
Pupal	0.71 and 1.75 w/cm², 3–18 min	161	1
Pupal	0.3 w/cm², 2–3 min	51	..
Pupal	0.71 w/cm², 25 min	89	..
Total		473	1
Eggs, larvae, pupae in controls		112	0

but experiments indicate that this possibility is remote.

In comparison with the resistance of *Drosophila* to x-rays and heat (Fig. 1), we found a very striking identity of effect by x-rays and by S.V. (*3*); while heat effects (*2*) do not resemble effects of S.V. To demonstrate the relation between survival rate and intensity, and/or time, we have to treat insects of identical age. A treatment with 0.71 and 1.75 w/cm² for 15–105 sec (Fig. 2) shows the importance of time; the relation is, however, not proportional. A comparable similarity to effects of x-radiation has been observed in modifications after S.V. treatment. Besides other variations (shortening of limbs, wings, etc.), a great number of treated late larvae and prepupae (in some cases even 20-hr pupae) died as late pupae, just before emergence, with a varying deficiency of chitin of the dorsal and ventral abdomen. The hypodermis was absent, while a normal pupal cuticle was present with brown spots, including the hypodermal defect. The same fact was observed by Bucher (unpublished data), when treating prepupae with x-rays, and it seems that x-rays and sonic waves of high frequency destroy the hypodermal histoblasts in the same manner. Other interesting abnormalities, like nonattachment of the testis to the vas efferens, will be described and discussed in a further paper.

During these experiments, we tried to discover whether S.V. could produce mutations. The imagoes derived from treated eggs, larvae, and pupae were examined for possible modifications, and were then used in crossing experiments in order to detect any recessive or dominant mutations. The CIB-method was applied for the X-chromosome, and, parallel to the experiment, we bred a control series, subject to the same procedure. In Table 2, we summarize some of the results of our experiments.

These first experiments seem to show that supersonic vibrations with an intensity of 0.3, 0.71, or 1.75 w/cm² do not produce many mutations. For a final answer, it would be necessary to know the effect of S.V. of small intensity or the effect upon dissected gonads, problems which we are studying further in our institute.

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A Simplified Method of Lyophilizing Microorganisms[1]

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The lyophilization of microorganisms is almost essential for their preservation without change of characteristics. It was shown to be effective for bacteria (*1–7*), for yeast (*8, 9*), and for fungi (*4*) and has been used satisfactorily for a number of years in our laboratories as a means of maintaining original and derived strains of many microorganisms. A simplified method developed in our laboratories for lyophilizing organisms, and in use for over a year, is herein described.

The method requires a minimum of special equipment and materials. A 0.1-ml sample (Fig. 1, A) of the spores, bacterial cells, or yeast cells in sterile skim milk or serum is introduced with a capillary pipette into a 12-in. length of 8-mm pyrex tubing sealed at one end and previously plugged loosely with nonabsorbent cotton and sterilized. This cotton plug (B) is pushed down the tube to a level 3½ in. above the sample, and 2–3 in. of the desiccant, powdered phosphoric anhydride (C), is added. A second and tight cotton plug (D) pushed down the tube wipes the loose P_2O_5 from the walls and holds the desiccant in place during evacuation. The P_2O_5 is introduced into the lyophil tubes from a dispenser such as that diagramed in Fig. 2. This device consists of a funnel turned on a lathe out of 2-in. brass stock; the lower end (Fig. 2, E) fits inside the lyophil tube and the upper end (F) is sealed with deKhotinsky cement to the open end of an inside section of a 45/50 standard taper pyrex interchangeable joint (G). The outside section of the joint (H) is fitted with a rubber stopper (I), carrying through the center a brass rod (J), which is long enough to be used as a plunger for ejecting the dry powdered P_2O_5. This apparatus is mounted in a ring stand. Enough P_2O_5 can be held in the dispenser when ¾ full for approximately 35 samples, a convenient unit of work.

After preparation of all the tubes for a given run the samples are frozen rapidly by immersing either in a solvent-dry ice mixture or in powdered dry ice alone. Each tube is individually evacuated by attaching with pressure tubing to a high vacuum electric pump. In order to keep the sample frozen during evacuation, an insulated shell vial containing powdered dry ice is held over the sample end of the tube. When evacuation is complete (usually in about 1 min or as indicated by a manometer), the tube, still under vacuum and in a horizontal position, is sealed with a flame[3] above the P_2O_5 (as indicated by

[1] This work was done in part under grants-in-aid from the American Cancer Society upon recommendation of the Committee on Growth, National Research Council, and from the Jane Coffin Childs Fund for Medical Research.

[2] The authors acknowledge the technical assistance of Dorothy Van Hacht and Carol Fuller.

[3] Hand gas-air torch, type 3A, National Welding Equipment Company, San Francisco, California, with tip size N-1.

an arrow in Fig. 1). Each tube is then placed in a rack with the sample immersed to a depth of 1 cm in a freezing mixture. A brine-ice bath or a glycol-water mixture, held at −5° C to −10° C in an open-topped refrigerator, can be used conveniently. The sample is completely dry in 2–4 hr. The lyophil tubes are removed from the freezing mixture and sealed off leaving about 2 in. of the tube above the sample (arrow in Fig. 1).

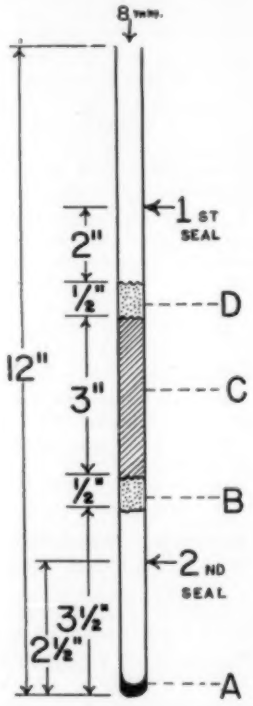

FIG. 1. Schematic drawing of a lyophil tube. A, Sample. B, First cotton plug. C, Phosphoric anhydride. D, Second cotton plug.

Several precautions should be observed: (1) For satisfactory desiccation the sample should not exceed 0.2 ml. (2) The glass surfaces to be sealed must be free of a film of P_2O_5. This is assured by the second cotton plug which wipes the surface. (3) Contact between P_2O_5 and moist air should be minimized, since it makes the compound sticky and extremely difficult to manipulate. (4) The P_2O_5 dispenser should be washed and thoroughly dried between each run.

In addition to the simplicity and low initial cost of the equipment, this method has the following advantages over the customary manifold method: (1) Each tube is evacuated independently, so that maintenance of a high vacuum in a large complicated glass system is obviated.

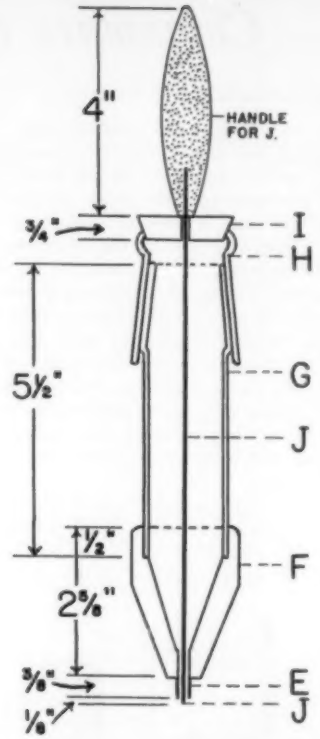

FIG. 2. Schematic drawing of the phosphoric anhydride dispenser. E, Brass tip which fits into lyophil tube. F, Brass shoulder of funnel, inside diam 1¼ in. G, Inside part 45/50 ST joint. H, Outside part 45/50 ST joint. I, Rubber stopper. J, Brass rod (¼ in.) which extends about ¼ in. beyond tip of dispenser when in lowest position.

(2) Any desired number of samples from a few to several dozen may be done at one time. (3) The lyophil tubes can be sealed while rotating in a horizontal position, and a structurally strong seal is therefore more easily made by an inexperienced worker. (4) The short distance between sample and desiccant speeds desiccation.

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Comments and Communications

Planchets for Radioactive Material

Radioactive materials are commonly put on or into flat or cupped planchets for convenience in handling and to obtain uniform conditions during activity measurements. These planchets are made of glass or some metal such as steel or copper. According to Albert Margnatti (*Tracerlog No. 22*, October, 1949) the best material for this purpose is one with the lowest possible atomic number, because backscattering is thus reduced. On this basis, aluminum becomes the material of choice.

In using planchets of glass or sheet metal, economy requires that they be cleaned and reused if possible. Much time and work could be saved if the planchet were disposable. After some experimentation, the writers have succeeded in producing planchets of aluminum foil which are so easily and cheaply made that they can be discarded after use.

The material finally selected was aluminum foil 0.001 in. thick. This can be purchased at grocery stores in rolls 1 ft wide and 25 ft long. Dies for forming the planchets were made as shown in Fig. 1. The upper

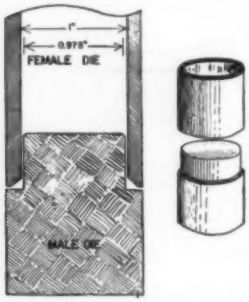

FIG. 1.

part is a 6-in. length of metal tubing with an inside diam of 1 in. The inner edge of one end is beveled slightly. The lower part is a 1¼-in. round steel bar with ¼ in. of the end turned down to 0.975 in. Again the sharp edge is beveled slightly. The loose fit permits crimping of the cup without tearing of the foil.

Aluminum foil disks 1¼ in. diam are cut on a sheet metal punch. By folding large strips of the foil into packs as many as 50 disks can be cut at once. After separation, these disks are placed on the die and formed. The crimped edges of the cup give it sufficient rigidity for most uses, and heavier cups can be made in the same dies by using two or more thicknesses of the foil, or heavier foil. Flat planchets must be made of foil at least 0.005 in. thick.

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Note on the Freezing Point of Citrate Solutions Used in the Dilution of Bull's Semen

In a recent paper on the freezing point of bull's semen and of the sodium citrate solutions used in diluting the semen for artificial insemination purposes, G. W. Salisbury, C. B. Knodt, and R. W. Bratton (*J. animal Sci.*, 1948, **7**, 283) have stated that "heating sodium citrate solutions increases their osmotic pressure. For heated solutions 2.9 grams of $Na_3C_6H_5O_7 \cdot 2H_2O$ per 100 ml of water distilled in glass is isotonic with blood." In view of the stable nature of sodium citrate, it appeared unlikely that the number of osmotically active particles should increase irreversibly when solutions of the salt were heat-treated. This point, which is of interest in connection with research in progress here on semen diluents, was, therefore, investigated by experiment.

Freezing points were determined by the Hortvet technique as used for milk in an improved model of the cryoscope developed at this institute (Temple, P. L. *Analyst*, 1937, **62**, 709). The thermometer had been standardized at the National Physical Laboratory, and all thermometer readings were corrected to the International Temperature Scale as recommended by R. Aschaffenburg and J. A. Hall (*Analyst*, 1949, **74**, 380). The freezing point of water was determined at the beginning and end of each set of tests.

EXPERIMENT 1

Solution A. $Na_3C_6H_5O_7 \cdot 2H_2O$ of reagent quality (2.9 g) was made up to 100 ml with glass-distilled water at 15° C.

Solution B. $Na_3C_6H_5O_7 \cdot 2H_2O$ (2.9 g) was dissolved in boiling distilled water, transferred with hot water (just off the boil) to a 100-ml measuring flask and, after cooling, made up to the mark.

Freezing point depressions in degrees Centigrade:

	Solution A	B
	0.525	0.525
	0.524	0.527
	0.525	0.525
Mean	0.525	0.526

EXPERIMENT 2

Solution C. Made up like Solution A, Experiment 1.

Solution D. Made up like Solution A, Experiment 1. The flask containing the solution was then immersed in boiling water for 6 hr, cooled to 15° C, and made up to the mark.

Freezing point depressions in degrees Centigrade:

	Solution C	D
	0.527	0.526
	0.525	0.527
	0.527	0.528
Mean	0.526	0.527

It must be concluded that heating sodium citrate solutions does not increase their osmotic pressure, and that solutions containing 2.9 g per 100 ml—whether they have been heated or not—are not isotonic with bull's blood which, according to Salisbury *et al.* (1948), has a mean freezing point depression of 0.56° C.

Nevertheless, in making up egg yolk-citrate diluents for semen, the use of solutions containing 2.9 g of $Na_3C_6H_5O_7 \cdot 2H_2O$ per 100 ml appears *theoretically* sounder than that of solutions containing 3.6 g (as earlier recommended by C. B. Knodt and G. W. Salisbury [*J. Dairy Sci.*, 1946, **29**, 285]) for which a freezing point depression of 0.645° C was found by the Hortvet technique. Since egg yolk has an average freezing point depression of 0.60° C (Needham, J., and Smith, M. *J. exp. Biol.*, 1931, **8**, 286), mixtures of equal parts of yolk and 2.9% citrate solution with a freezing point depression of 0.526° C are likely to be nearly isotonic with fresh bull's semen which has an osmotic pressure equal to, or slightly higher than, bovine blood (Salisbury *et al.*, 1948). Mixtures of equal parts of yolk and 3.6% citrate solution, on the other hand, are clearly hypertonic to semen. Whether this point is of *practical* importance, however, remains to be seen. The good results of artificial insemination obtained in the past with 3.6% citrate solution suggest that, within limits, bull's semen is not very sensitive to variations in the tonicity of the diluent.

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A Simple Method for Opening Quartz Capsules Containing Radioactive Materials

The authors encountered considerable difficulty in opening capsules containing either irradiated red phosphorus or phosphorus pentasulfide obtained by service irradiation at Oak Ridge National Laboratories on authorization from the Atomic Energy Commission. These materials were submitted for service irradiation sealed in quartz capsules of less than 3 in. in length and ¼ in. in diam, packed under nitrogen at less than atmospheric pressure. The phosphorus was obtained in 1-g lots with an activity of 50 mc, and the phosphorus pentasulfide was obtained in a 4-g sample with an activity of 150 mc.

The apparatus shown in the accompanying drawing was used to open the sample of P_2S_5 without any laboratory or personnel contamination and also served to minimize the exposure of the operator's hands. (The survey meter read less than 2 mr/hr outside the case.)

After unpacking, the quartz capsule (Fig. 1, *4*) was placed in a hole of the proper dimensions in a lucite rod (Fig. 1, *5*) 1½ in. × 2½ in. A cement of lucite dissolved in chloroform was used to secure the capsule in the lucite rod. This lucite rod was placed in a lucite box (Fig. 1, *7*) 4 in. × 4 in. × 4 in. with walls ¼ in. thick. This box was equipped with a retaining screw for holding the lucite rod and with a filter packed with glass wool (Fig. 1, *8*) to remove any radioactive materials that might be dispersed when the capsule was opened. The filter was connected to a vacuum source to insure a flow of air into the box through the shaft opening and at the junction between the cover and the box. The sliding cover (Fig. 1, *2*) was of ¼-in. lucite, 6 in. × 6 in. A Dremel Moto Tool, or equivalent, was mounted on the sliding cover with the shaft projecting into the box. A carborundum cutting disk (Fig. 1, *3*) was mounted on the shaft, which was positioned in the chuck so as to bring the cutting disk in proper alignment with the quartz capsule. The cover was then placed on the box slightly off center so as to bring the edge of the carborundum disk into contact with the side of the quartz capsule. The sliding cover was then moved slowly so that the carborundum disk cut a groove completely around the capsule.

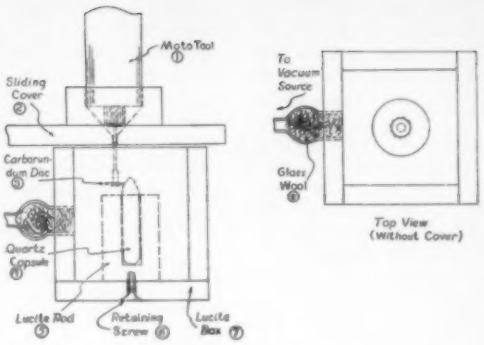

FIG. 1.

The authors have found it desirable to cut the top off completely with the cutting tool rather than break it at the groove by a sharp blow, since the sudden rush of air into the capsule tends to disperse the radioactive material. After the top is sawed off, the retaining screw is loosened and the lucite rod is removed with tongs to transfer the radioactive materials to the reaction flasks.

For gamma emitters the box and sliding lid can be constructed of lead, with lucite employed for the side of the box away from the operator in order to provide light. A lead-glass observation window in the front or a mirror placed behind the box would enable the operator to observe the position of the carborundum disk and the progress of the cutting operation.

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Water-soluble Riboflavin Derivative

The recent paper "A Very Water-soluble Riboflavin Derivative" by George B. Stone (*Science*, 1950, **111**, 283) prompts us to report our own findings, which have a relation to his. During our search for water-soluble, biologically active riboflavin derivatives, which resulted in the preparation of methylol derivatives of riboflavin (Schoen, K., and Gordon, S. M. *Arch. Biochem.*, 1949, **22**, 149) we prepared in 1947 a riboflavin sulfate ester by essentially the same method as reported by Stone.

Five grams of riboflavin, suspended in 10 ml water, was cooled to 0° C in an ice-salt mixture, and 50 ml concentrated sulfuric acid was added dropwise with stirring at such a rate that the temperature remained between −5° and +10° C. After stirring for 30 min, the solution was poured on 500 g ice and brought to pH 2 with solid barium hydroxide. Sodium bicarbonate was added to pH 7 and the solution was concentrated *in vacuo* to 50 ml. A sodium salt of the riboflavin ester was precipitated by addition of 500 ml anhydrous ethanol and 200 ml acetone. A yellow, very water-soluble powder was obtained.

A solution of this product containing the equivalent of 6 mg riboflavin per milliliter had a microbiological activity of 0.25 mg riboflavin per mill liter, an activity of about 4%. This compares with an activity of 1.5% for Stone's product. Under the conditions of our experiment, it is probable that mainly the primary hydroxyl group of riboflavin has been esterified (Swern, D., *et al. Oil and Soap*, 1943, **20**, 224). As in the case of tri- and tetrasuccinates (Furter, M. F., Haas, G. T., and Rubin, S. H. *J. biol. Chem.*, 1945, **160**, 295), the sulfate is biologically active only after previous hydrolysis by autoclaving. In contrast to this behavior, our methylol derivatives are active biologically without previous hydrolysis, and autoclaving does not increase their activity.

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Erratum

In our paper "Pantothenic Acid in Copper Deficiency in Rats" (*Science*, 1950, **111**, 472), in the last sentence of the second paragraph on page 473, the daily administered dose mentioned should be "0.1 μg of copper" and not "1.1 μg" as printed.

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Foreign Publications in the Field of Organic Chemistry

I have read with great interest the article by F. S. Boig on domestic and foreign periodicals in the field of organic chemistry (*Science*, 1949, **110**, 107). Boig tabulated (see Table 2, page 108) the number of organic chemical publications in various countries in the years 1937 and 1947, measuring the organic chemical research in these countries by the volume of organic chemical publications and drawing certain conclusions from the results.

One arrives at even more interesting conclusions if one does not simply compare on this basis such very large countries as the United States and Russia with such very small countries as Holland, Finland, Sweden, and Switzerland, but instead computes the ratio of the number of inhabitants to the number of organic chemical papers in these countries.

TABLE 1

	Country*	Year	No. of inhabitants for each organic chemical publication	Rank
I.	Switzerland	1937	42,000	1
		1947	22,000	
II.	Germany	1937	112,000	2
	Finland	1947	125,000	3
	U.S.A.	1947	135,000	4
	British Isles	1947	139,000	5
	Holland	1947	150,000	6
	France	1947	168,000	7
	Sweden	1947	179,000	8
III.	Italy	1937	275,000	9
	Austria	1937	350,000	10
	Japan	1937	390,000	11
	U.S.S.R.	1937	440,000	12

* Category I: less than 50,000 inhabitants per publication.
" II: 100,000–200,000 " " "
" III: 300,000–500,000 " " "

Thus the countries are grouped here in Table 1 in a somewhat different manner than in Boig's table. Switzerland now achieves a unique position, far ahead of the other categories.

For several of the countries I have chosen the number of papers from the year 1937 instead of 1947, since it is certainly not logical to take for comparison the strongly war-exhausted countries like Germany and others with the greatly reduced production of the year 1947. It is perhaps also of interest to point out further that, for the time of the organic chemical *Hochkonjunktur* in Germany about 1910, this country would fall in category I.

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Use of Dried Hemoglobin in the Assay of Pepsin

A recent communication in *Science* (Orringer, D., Lauber, F. U., and Hollander, F. *Science*, 1950, **111**, 88) demonstrates the feasibility of substituting lyophilized bovine hemoglobin powder (Armour) for hemoglobin prepared from fresh blood in the assay of pepsin and trypsin by the well-known method of Anson and Mirsky (Anson, M. L., and Mirsky, A. E. *J. gen. Physiol.*, 1932, **16**, 59). The author many years ago demonstrated that dried hemoglobin "scales," as available commercially, could be used in a very simple assay of pepsin by a very slight modification of the original method which improved its accuracy (Steinhardt, J. *Kg. Danske Videnskab. Selskab., Math-fys. Medd.*, 1937, **14**, No. 11, 1; *J. biol. Chem.*, 1939, **129**, 135).

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Book Reviews

The Chemical Elements and Their Compounds, 2 vols. N. V. Sidgwick. New York: Oxford Univ. Press, 1950. 1703 pp. $14.00 the set.

The title *The Chemical Elements and Their Compounds* implies a comprehensive work, and the author has written a book to which that term certainly applies. This reviewer stands in awe of any one man who can "attempt to discuss in detail the properties of the elements and their compounds in the light of modern ideas of atomic and molecular structure." This is accomplished in 1,700 pages, which are divided into 25 chapters titled according to the groups and subgroups in the periodic table, and hence running from the chapter covering Group O, the inert gases, to Group VIIIC, palladium and platinum. The comprehensiveness of the book is illustrated by the fact that some 65 pages of "organic chemistry" appear in the chapter covering Group IV, in view of the position of carbon in this group (rather than in the chapter covering Group I, which includes hydrogen).

The most dependable books in the various fields of science seem to be those written by the actual participants in those fields. A yardstick based on this standard is clearly not justified in evaluating a book which covers all of the chemical elements. The value of such a book lies in putting in one place a broad range of essential and concise information, and it would be difficult to make it authoritative in all details. The present book is noncritical in numerous instances, giving incorrect along with correct information; since the information is so well documented with references it may have been the author's aim to do this intentionally, in emulation of the famous treatise by Mellor. It is, of course, inevitable that much of the information and interpretation considerably predates the publication date of 1950.

Perhaps the most surprising shortcoming of the book is the almost complete lack of the thermodynamic approach. Discussions in terms of oxidation potentials are almost completely lacking. Consideration of questions in the light of modern ideas of atomic and molecular structure, as promised in the preface, is not as extensive as one might have expected from the author of *The Electronic Theory of Valency.*

Criticism of details can only serve as examples. There are numerous small errors, as exemplified by the listing of the half-life of the neutron as infinite. The most important method for the separation of the rare earth elements from each other, the ion-exchange adsorption method, is not mentioned at all, even though most of the important wartime work was published as early as 1947. Also, the electronic structures given for the rare earth elements are not those that have been generally accepted for many years. The treatment of the transuranium elements and those immediately preceding them is probably the most confused in the book. These appear as members of the chromium group and are first discussed on this basis in a treatment which gradually veers over to the point of view that a rare earthlike transition series is involved. Thus curium is both a homologue of chromium and is explicitly said to have an electronic structure analogous to gadolinium.

In balance, however, the book, in the opinion of this reviewer, is valuable and probably an almost indispensable adjunct to the library of most chemists from the standpoint of its central purpose—that of an over-all and broad reference book. Many readers will feel that the 10,000 references to the original literature are of sufficient value to make the book worth owning regardless of its other merits.

GLENN T. SEABORG

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Elementary Pile Theory. Harry Soodak and Edward C. Campbell. New York: John Wiley; London: Chapman & Hall, 1950. 73 pp. $2.50.

In rereading the notes of Harry Soodak's lectures on elementary pile theory I am reminded of the happy days of 1946–47, when a training school on nuclear physics and "atomic" energy was organized at the Clinton Laboratories with the intent of spreading for the public benefit some of the knowledge accumulated by the Manhattan Project during the war. Those were days of hope, when one thought that atomic energy would enrich mankind and unite the world.

Soodak's lectures were one of the high lights of the training program. They were attended mostly by engineers who expected to apply the acquired knowledge in their industrial work. Excellent notes were compiled by E. Campbell and distributed in dittoed form within the AEC. Now they are available to the public in book form. The few omissions imposed by security do not affect the continuity of treatment.

The text should prove very useful, as the lectures were, to anyone wishing to learn how a nuclear reactor works. Wisely, it does not discuss nuclear physics and does not require any knowledge of quantum mechanics.

Neutrons are considered as particles wandering through matter, following laws similar to those which govern the motion of the molecules of a gas. Their collisions with nuclei are described in terms of cross sections, whose theoretical interpretation is not given, but whose definition is clear and easily understood. The consequences of this approach are developed in a logical and well-organized manner, making the book more similar to a simple treatise on mathematical physics than to an engineering manual. In the first half the slowing down (moderation) and the diffusion of neutrons are discussed. The second half is devoted to reactors proper and includes such topics as critical size, transient behavior, and control.

If the engineering details, as well as some of the pertinent numbers, are missing, the basic principles of pile physics are all there. The reader who is interested in

"understanding" will satisfy his curiosity. The engineer who is interested in "using" (and who has access to more detailed information) will find this book a good introduction to the reading of technical papers.

S. DEBENEDETTI

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The Theory of Atomic Collisions. 2nd ed. N. F. Mott and H. S. W. Massey. New York: Oxford Univ. Press, 1949. 388 pp. $8.75.

The publication of a new edition of *The Theory of Atomic Collisions* would seem to call not so much for the customary critical review as for a formal expression of appreciation in behalf of the many whom it will doubtless serve so well. The first edition, invaluable in the education and activities of physicists since its appearance in 1933, has distinguished itself by its usefulness to theoretician and experimentalist alike; it is *the* reference on the subject in classroom, laboratory, and library. The book is a systematic exposition of the applications of quantum mechanics to collision problems—problems ranging from the scattering of ultrahigh-energy mesons, which provides information on the nature of elementary particles, to collisions between atoms at thermal energy, the treatment of which leads to the quantum theory of viscosity and diffusion. The scope is exhaustive, the development authoritative, the style lucid and incisive; moreover, the volume is singularly rich in its evocation of experimental results. Although the first edition is in no sense obsolete, the new version is greatly augmented in value, for it encompasses almost the whole of the old and a wealth of new material—later applications and new techniques of the theory as well as some advances in the theory itself; and this without unduly increasing the size of the book.

The most important addition is certainly that of extensive material on nuclear collisions. Specific major changes are: amplification of the treatment of scattering by a potential well and barrier; inclusion of the "dispersion" method and a variety of its applications; addition of much new material on the nuclear scattering of electrons and positrons; discussion of new methods for treating scattering by a central force and a more detailed analysis of the conditions for validity of the older methods; greatly extended consideration of "slow" collisions; addition of material on multiple scattering of electrons; a new chapter on nuclear collisions—neutron and charged particle transmutations, fission, and scattering, with an ample section on the scattering of slow neutrons (including scattering by bound atoms and magnetic scattering); extension of the final chapter, which deals with relativistic problems—largely by consideration of positron and meson processes. The chapter on collisions of electrons with molecules is deleted, but in compensation we are promised a new book, *Electronic and Ionic Impact Phenomena*, by Massey and Burhop, which is now in preparation and will deal in much greater detail with this and related topics.

It may be reassuring to many that this volume, coming as it does at a time when much of our basic theory is in a state of bewildering—not to say disillusioning—difficulty, reaffirms our confidence in the quantum mechanics by its account of that theory's long succession of triumphs in dealing with a great and remarkably diverse set of phenomena.

ROBERT L. PLATZMAN

Purdue University

Scientific Book Register

Advances in Colloid Science, Vol. III. H. Mark and E. J. W. Verwey, eds. New York-London: Interscience, 1950. 384 pp. $7.50.

A Geography of Europe. Jean Gottmann. New York: Henry Holt, 1950. 688 pp. $5.00.

College Chemistry: An Introductory Textbook of General Chemistry. Linus Pauling. 705 pp. $4.50. ***College Chemistry in the Laboratory: A Manual Designed to Accompany Pauling's College Chemistry.*** Lloyd E. Malm and Harper W. Frantz. 331 pp. $3.00. San Francisco, Calif.: W. H. Freeman, 1950.

A Histology of the Body Tissues with a Consideration of Their Functions. Margaret Gillison. Baltimore, Maryland: Williams and Wilkins, 1950. 220 pp. $3.50.

History of the Primates: An Introduction to the Study of Fossil Man. 2nd ed. W. E. Le Gros Clark. London: British Museum (Natural History), 1950. 117 pp. 2/6.

General Chemistry. 4th ed. H. I. Schlesinger. New York: Longmans, Green, 1950. 811 pp. $5.50.

Primary Batteries. George Wood Vinal. New York: John Wiley; London: Chapman & Hall, 1950. 336 pp. $5.00.

Vorstufe zur Theoretischen Physik. Richard Becker. West Berlin, Germany: Springer-Verlag, 1950. 172 pp. DM 7.50.

Tissue Culture Technique. Rev. 2nd ed. Gladys Cameron. New York: Academic Press, 1950. 191 pp. $4.20.

Marriage and Family Relationships. Rev. ed. Robert Geib Foster. New York: Macmillan, 1950. 316 pp. $2.75.

The Laboratory Guide in Chemistry. 2nd ed. Joseph H. Roe. St. Louis, Mo.: C. V. Mosby, 1950. 216 pp. $2.25.

The James River Basin: Past, Present and Future. Compiled by the James River Project Committee, Virginia Academy of Science, 1950. 843 pp. Order through Foley F. Smith, Box 1395, Richmond 11, Virginia. $6.00 postpaid.

Principles of Chemistry: An Introductory Textbook of Inorganic, Organic, and Physiological Chemistry for Nurses and Students of Home Economics and Applied Chemistry. 7th ed. Joseph H. Roe. St. Louis, Mo.: C. V. Mosby, 1950. 427 pp. $3.50.

An Introduction to Experimental Stress Analysis. George Hamor Lee. New York: John Wiley; London: Chapman & Hall, 1950. 319 pp. $5.50.

News and Notes

About People

Emerson Day, associate professor of public health and preventive medicine at Cornell University Medical College, has been appointed director of the Kate Depew Strang Cancer Prevention Clinic, a part of Memorial Center for Cancer and Allied Diseases, in New York City. Dr. Day will assume his post September 1.

Glenn Glauser has been appointed director of development of Columbia University's College of Physicians and Surgeons. Dr. Glauser was former director of government liaison and special services for the Community Chests of America and the United Service Organizations, Inc.

Franz Newell Devereux Kurie and **John Milton Miller** have been appointed special consultants to E. O. Hulburt, director of research at the Naval Research Laboratory. Dr. Kurie, at present superintendent of the Nucleonics Division, will plan research programs in the field of nuclear physics and nucleonics. Dr. Miller, superintendent of Radio Division I, will serve as consultant in electronic research and development.

William deBerniere MacNider, Kenan Research Professor of Pharmacology at the University of North Carolina, retired July 1. Dr. MacNider had been a member of the faculty of the School of Medicine for 51 years.

L. Corsan Reid has been appointed as professor of experimental surgery at the New York University Post-Graduate Medical School, a unit of the New York University-Bellevue Medical Center. Dr. Reid has served since 1946 as an associate professor of physiology at the university's College of Medicine.

Tufts College Medical School, Boston, has appointed **Henry Sable,** of the University of Toronto, and **Thomas R. Riggs** as instructors in the Department of Biochemistry and Nutrition.

J. Leon Shereshefsky, professor and head of the Chemistry Department of Howard University, Washington, D. C., will serve as visiting professor of physical chemistry at the Hebrew Institute of Technology, Haifa, Israel, for the academic year 1950–51.

Gerald F. Tape, formerly associate professor of physics at the University of Illinois, has joined the scientific staff of Brookhaven National Laboratory to assist Leland J. Haworth, director. Dr. Tape worked on radar development at the Malvern, England, branch of Massachusetts Institute of Technology's Radiation Laboratory during World War II.

Emory University, Atlanta, Georgia, recently made five new appointments to its science staff. **Alfred E. Wilhelmi,** formerly of Yale University, will head the Department of Biochemistry, and his wife, **Jane A. Russell,** will be assistant professor of biochemistry. **Rolla Eugene Dyer,** director of the Division of Infectious Diseases, U. S. Public Health Service, has been appointed director of research in the university's Winship Clinic. Other appointments in the School of Medicine are **Richard E. Boger,** assistant in pediatrics, and **Bernard S. Lipman,** assistant in medicine.

William S. Wilson, head of the Department of Chemistry, University of Alaska, has been appointed acting director of the university's Geophysical Institute, College, Alaska.

Visitors

R. T. Bell, of the Department of Chemistry, Balliol College, Oxford University, England, visited Iowa State College's Institute for Atomic Research, June 26–29.

The Connecticut State Department of Health recently has had as visitors the following: **Guillermo Adriazola,** who has been a health officer in Chile, and who expects to take over maternal and child health services within the National Health Insurance Program on his return to that country; **Ruperto Angodung,** district health officer, of Bacolod, Negros, Occidental, the Philippines; **Masayasu Kusumoto,** chief of the Health Center Section, Public Sanitation Bureau, Japanese Ministry of Welfare; **Eyvind Ek,** medical officer with the Oslo, Norway, Department of Health, and assistant director of Oslo's maternal and child health programs; **Oscar C. Chacon** and **Gerardo B. Neri,** of the Philippines; **Nouhad N. Beyhum,** Lebanon, who will return to the Lebanese Ministry of Health, where he will be in charge of communicable diseases; and **Hildegard Rothmund,** Germany, who will return to that country as a country health officer.

Grants and Awards

The American Chemical Society's Western New York Section has given the 1950 Schoellkopf Medal to Joseph Harrison Brennan, chief metallurgist, Electro Metallurgical Division, Union Carbide and Carbon Corporation, in recognition of his contributions to the metallurgical practices of the ferro-alloy industry.

Awards for 50 new projects were included in **National Cancer Institute** grants totaling $1,160,818 for support of cancer research in hospitals, universities, and other nonfederal institutions. The following institutions received grants of $10,000 or more: Southern Research Institute, Birmingham, Alabama, $14,688; Santa Barbara Cottage Hospital Research Institute, Santa Barbara, California, $10,888; Emory University School of Medicine, Georgia, $13,176; Louisiana State University, Baton Rouge, $11,804; Massachusetts Institute of Technology, Cambridge, $22,000; University of Minnesota, Minneapolis, three grants totaling $29,748; University of Missouri, Columbia, $10,000; Washington University, St. Louis, two grants

totaling $17,621; Rutgers University, New Brunswick, New Jersey, $15,000; New York State College of Agriculture, Ithaca, $11,000; Memorial Cancer Center, New York City, three grants totaling $36,000; University of Rochester, Rochester New York, two grants totaling $13,524; University of Pennsylvania, Philadelphia, three grants totaling $31,509; and University of Tennessee, Memphis, three grants totaling $34,146.

Henry G. Booker, professor of electrical engineering at Cornell University, and P. C. Clemmow, of the University of London, have been given the **Kelvin Premium for 1950** of the Institution of Electrical Engineers, London, for two papers in the field of wave propagation.

At its annual convention in June, the **American Medical Association** presented its highest honor—the distinguished service award—to Evarts A. Graham, professor of surgery at Washington University, St. Louis. Dr. Graham is known for his pioneer work in gall bladder disease and in lung surgery. The AMA gold medal for original research went to Lester R. Dragstedt and his associates, from the University of Chicago, for their special operative technique used in the study of the secretion of gastric juices. The silver medal was given to Robert Elman and his team of research workers from Washington University, St. Louis, and the bronze medal went to A. C. Ivy and Louis R. Krasno, University of Illinois.

Eight European women have been given international scholarships and fellowship awards by the **American Home Economics Association**, to study home economics in cooperating U. S. colleges and universities during the academic year 1950–51. Recipients and the institutions at which they will study are: Margaritha Glotz, Vienna, Austria—University of Wisconsin; Eileen M. Herrington, London—Rhode Island State College; Tuovi E. A. Kanninen, Jyväskylä, Finland—Ohio State University; Toini E. Tuomikoski, Kaarela, Finland—University of Illinois; Elisabeth M. Engelken, Frankfurt, Germany—Texas State College for Women, Denton; Nomiki Tsoukala, Athens, Greece—Winthrop College, Rock Hill, South Carolina; Theodora F. S. M. van Schaik, The Hague, Netherlands—Michigan State College; and Elsa G. Haglund, Stockholm, Sweden—Pennsylvania State College. Miss Haglund has been designated the association's 1950–51 Helen W. Atwater international fellow, and will receive an additional grant.

Colleges and Universities

The **University of California** at Berkeley has received from Hermann O. L. Fischer, of the Department of Biochemistry, the complete working library of his father, Emil Fischer, an early researcher in biochemistry. The collection, which will be the nucleus of a library in the virus laboratory and biochemistry building soon to be constructed at Berkeley, contains a particularly comprehensive group of periodicals covering the fields of chemistry and biochemistry. Many of the sets are complete from the first mid-nineteenth century issue to the present.

The **University of Wisconsin** has established a Department of the History of Medicine in its Medical School. The new department will be headed by Erwin H. Ackerknecht, who came to the university in 1947 as its first professor of the history of medicine.

Florida State University is offering graduate assistantships in the Department of Meteorology for the academic year 1950–51. Minimum requirement for an assistant is a bachelor's degree including two years of mathematics and one year of physics. The stipend is $125 a month, and students are not required to pay out-of-state tuition. Applications must be filed with the Dean of the Graduate School, Florida State University, Tallahassee, not later than *August 12*.

Several staff members of the **California Institute of Technology** are spending part of the summer in Europe, attending conferences or conducting research. Fritz Went, professor of plant physiology, and Arthur W. Galston, senior research fellow in biology, attended the International Botanical Congress at Stockholm in July. C. A. G. Wiersma, professor of biology, and A. Van Harreveld, professor of bio-organic chemistry, will attend the International Physiological Congress in Copenhagen, August 15–18. Albert E. J. Engel, associate professor of geology, will attend the 25th anniversary meeting of the Mineralogical and Petrological Institute in Switzerland in August. David Lind, senior research fellow in physics, will go to Stockholm in September, where he will study under a Guggenheim Fellowship at the Nobel Institute of Physics.

Industrial Laboratories

The E. I. du Pont de Nemours & Co., of Wilmington, Delaware, is making a special survey covering facilities, processes, and technical problems in certain fields of chemical and reactor engineering. The work involves analysis of a number of existing and planned projects in the atomic energy development program including process feasibility studies, developmental projects, and facilities requirements.

Sharp & Dohme, Incorporated, has made two recent appointments to its medical staff: W. E. Askue, who has just completed a 12-month residency in pediatrics at the Norwegian Hospital, Brooklyn, will conduct clinical investigation on new products in the company's Medical Division, at Philadelphia. R. E. Bauer, formerly plant physician at the Glenn L. Martin Company in Baltimore and assistant in pathology at the Maryland University School of Medicine, and more recently resident at the hospital of the School of Medicine, will serve as medical director of the Sharp & Dohme Blood Donor Center, at Baltimore.

The Acetate Research Laboratory of the Du Pont Company's Rayon Department at Waynesboro, Virginia, has been named **Benger Laboratory** in honor of Ernest B. Ben-

ger, former manager of the Rayon Technical Division, who retired three years ago, after 30 years as a research chemist with the company.

Meetings and Elections

The Robert Gould Foundation of Cincinnati will sponsor a symposium on the ***Biological Significance of Lipids***, September 13–14, at the University of Rochester School of Medicine and Dentistry. The symposium will be the third in an annual series which the foundation sponsors in the field of nutrition.

The American Astronomical Society elected the following officers at its June 21 meeting in Bloomington, Indiana: vice president (1950–52), C. S. Beals, Ottawa, Canada; secretary (1950–51), C. M. Huffer, Madison, Wisconsin; treasurer (1950–51), J. J. Nassau, East Cleveland, Ohio. Alfred H. Joy, Mount Wilson Observatory, Pasadena, California, remains as president.

The Mississippi Academy of Science elected the following officers for the ensuing year: president, Richard R. Priddy, Millsaps College; vice president, A. B. Lewis, University of Mississippi; and secretary-treasurer and executive officer, Clyde Q. Sheely, Mississippi State College.

Deaths

Alfred Meyer, 96, died July 14 at his summer home in Ogunquit, Maine. Dr. Meyer, a specialist in diseases of the lungs, was one of the founders of the National Tuberculosis Association.

Charles Frederick Bolduan, bacteriologist, died July 5, after a brief illness. He was 77. Dr. Bolduan was a public health official in New York City for many years, and in 1913 he established the Bureau of Health Education. He was also a founder of the American Diabetes Association.

Henry Cuthbert Bazett, professor of physiology at the University of Pennsylvania, died July 11, aboard the ***Queen Mary***, on his way to the International Physiological Congress in Copenhagen. He was 65. Dr. Bazett, a member of the university's faculty since 1921, was an authority on climatology, and had conducted research on undersea temperature control for the U. S. and British governments during World War II.

Otto A. Beeck, 45, associate director of research at Shell Development Company, Emeryville, California, died July 5. Dr. Beeck, a member of the AAAS, had done research on catalysis, the physics and chemistry of surface phenomena, reaction mechanisms, using tracer methods, and fundamentals of lubrication.

John L. Smith, chemist, and chairman of the board of Charles Pfizer & Co., Inc., died July 10, at the age of 61. Dr. Smith had worked on the development of commercial production and improvement of penicillin.

Miscellaneous

A technical film of the life cycle of ***Diphyllobothrium latum***, the fish tapeworm, has been prepared by the Audio-Visual Production Services of the U. S. Public Health Service's Communicable Disease Center at Atlanta. The first public showing of the film, which is intended for public health scientists and laboratory workers, will be at the Fifth International Congress of Microbiologists in Rio de Janeiro, August 17–24.

Declassified and unclassified atomic energy research reports are available in 31 libraries designated as official depositories by the Atomic Energy Commission. Approximately 3,500 reports comprise a full set, and about 1,500 reports are issued each year. The reports, as well as a card catalogue index, will be furnished by the AEC, and the libraries will provide access to the reports, reference service, and photocopies to users. The libraries receiving these materials are: University of California at Berkeley; University of California, Los Angeles; Denver Public Library, Colorado; Yale University; Library of Congress; Georgia Institute of Technology, Atlanta; University of Chicago; John Crerar Library, Chicago; University of Illinois; Iowa State College, Ames; Louisiana State University, Baton Rouge; Harvard University; Massachusetts Institute of Technology; University of Michigan; Detroit Public Library; University of Minnesota; Linda Hall Library, Kansas City, Missouri; Washington University, St. Louis; Princeton University; Cornell University; Columbia University; New York City Public Library; Duke University; Cleveland Public Library; Ohio State University; University of Pennsylvania; Carnegie Library of Pittsburgh; Joint University Libraries, Nashville; University of Texas; University of Washington; University of Wisconsin.

The 1950 Bermuda Zoological Expedition of the Chicago Natural History Museum has begun undersea work from the Bermuda Biological Station for Research, on St. George's. Fritz Haas, curator of lower invertebrates, leader of the expedition, will be assisted by Joseph B. Krstolich, artist in the Department of Zoology. Collecting and research will be carried on for two months.

The New York Academy of Medicine on July 27 began broadcasting a **Post Graduate Radio Program** to make information on medical progress available to physicians in the New York City area. The programs, which will be broadcast over Station WNYC-FM from 9:00 to 10:00 p.m. each Thursday, will consist of selected lectures on current developments in medical science, delivered before the academy and its affiliated organizations. Communications concerning the programs to be presented or for further information should be addressed to Iago Galdston, Executive Secretary, Committee on Medical Information, 2 East 103rd Street, New York City.

A survey of plague in Venezuela is being conducted by the Pan American Sanitary Bureau, Regional Office of WHO, at the request of the Ministry of Health. A study unit,

headed by Julius M. Amberson, of the Bureau of Medicine and Surgery, U. S. Navy, will make an epidemiological rodent and insect survey of the endemic area of the Campamento Rafael Rangel. Ernst Schwarz, of the Bureau of Medicine and Surgery, will assist Commander Amberson in the eight-week study.

A fisheries research laboratory has been established in Honolulu, Hawaii, by the Fish and Wildlife Service of the U. S. Department of the Interior. The laboratory, headed by Oscar E. Sette, will conduct research supplementing or paralleling the exploratory investigations being carried on at sea by three vessels of the Pacific Oceanic Fishery Investigations. Through this research and experimentation, investigators will develop and coordinate the basic information for a productive American fishing industry in the Pacific.

A survey of the incidence of infection among laboratory and research workers is being conducted by S. E. Sulkin and R. M. Pike, of Southwestern Medical College, University of Texas. This survey, aided by a grant from the National Institutes of Health, PHS, will compile data on the numbers and types of infections which have occurred in the past 20 years in governmental and private laboratories handling infectious agents. The results of the survey, including information on protective measures, will be presented at the annual meeting of the American Public Health Association in St. Louis, October 30–November 3.

Aboard the laboratory ship *Megalopa*, Dr. Hilary B. Moore, associate director of the National Geographic–University of Miami plankton research expedition, helps an assistant attach a bathythermograph to the seining equipment. In addition to exploration of the ocean depths for the microscopic plankton, new information will be sought on the behavior of the Gulf Stream. Dr. F. G. Walton Smith, head of the Marine Laboratory, University of Miami, is director of the expedition, which left Miami July 25.

The Army Medical Library has prepared a ***Catalogue of Incunabula and Manuscripts in the Army Medical Library,*** listing and describing the library's holdings of three classes of books. The first part of the book lists the incunabula; the second, early Western manuscripts (Latin, German, Italian, and English); and the last, Oriental manuscripts (Arabic, Persian, Turkish, Singhalese, and Hebrew). The catalogue, compiled by Dorothy M. Schullian and Francis E. Sommer, was published by Henry Schuman, Inc., New York City.

A manual of cerebral palsy equipment, containing information regarding use, diagrams for construction, specifications, materials, and directions for making 127 items of equipment, has been completed by the National Society for Crippled Children and Adults. This manual, compiled with the assistance of the American Academy for Cerebral Palsy and other agencies, will aid in construction of equipment that must be designed for the individual patient's needs. It is available from the National Society for Crippled Children and Adults, 11 South La Salle Street, Chicago.

Prevention of Deterioration Abstracts, published by the Prevention of Deterioration Center of the National Research Council, 2101 Constitution Avenue, Washington, D. C., are offered for subscription on a yearly basis. Abstracts are classified under the headings: Biological Agents; Electrical and Electronic Equipment; Fungicides and Other Toxic Compounds; Lacquers, Paints, and Varnishes; Leather; Lubricants; Metals; Miscellaneous; Optical Instruments and Photographic Equipment; Packaging and Storage; Plastics, Resins, Rubbers, and Waxes; Textiles and Cordage; and Wood and Paper. One volume is published each year in monthly issues. Subject and author indexes are compiled annually to cover abstracts issued from July through June. The yearly rate of $50 includes two binders and index tabs. An *Advance List,* a monthly bibliography of all reports received in this field, is available for $10 a year.

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POSITIONS WANTED

Academic position desired by Ph.D. (Columbia University, 1949) in Education and Philosophy, with broad training in related fields. One year of active teaching experience. Four years of research and translation experience from English to Chinese. Can speak and write both Chinese and English fluently. Box 233, SCIENCE. 8/11

Bacteriologist: woman Ph.D., age 33, with teaching and research experience, bacterial genetics, general and medical bacteriology, desires academic or research position. Box 248, SCIENCE. X

Biological or medical research position desired, industrial or otherwise; veteran, 33, B.S. Chemistry, M.S. Biology, 3 years pre-doctoral in anatomy, pathology, other interests physiology, parasitology; publications. Box 244, SCIENCE. X

Biophysicist: Ph.D. Age 28. Broad background in physics and biology, specializing in electro-physiology. Full time research or half-time teaching. Box 241, SCIENCE. X

Biophysicist: Desires teaching and research in medical school or college. Ph.D. candidate 1950. 2 years experience in nuclear physics. 1½ years in medical physics. 1½ years in neuro-physiology. Research interest in neurophysiology. Publications. Box 249, SCIENCE. X

Entomologist, M.S., Field and teaching experience. Desires research or technical sales position. Box 236, SCIENCE. 8/4

Instrumentation position in medical physics desired by Electrical Engineer with M.S. in Zoology, having finished credits for Ph.D. Designed, radio cardiography apparatus supersonics, scintillation counters, also teaching experience in Zoology. Papers. Box 245, SCIENCE. X

Microbiologist, M.Sc., woman, degree minors—chemistry, soil bacteriology, research experience. Box 228, SCIENCE. 7-28

Physiologist: Ph.D.; two years, instructor, physics and two years, research associate, Middle Western University; four years, assistant professor, physiology, university medical school; for further information, please write Burneice Larson, Medical Bureau, Palmolive Building, Chicago. X

Plant Pathologist: Ph.D. September, 1950, desires university teaching-research position starting fall. Box 246, SCIENCE. X

Zoologist: 30 M.S. desires position teaching, research. Fellowship teaching and assisting experience in Protozoology, Zoology, General Physiology. Considerable work toward Ph.D. Box 242, SCIENCE. X

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The Market Place

BOOKS

The Market Place

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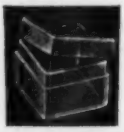

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